46905

Access DB# _____

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Natalie Davis Examiner #: 78462 Date: 7/6-01
Art Unit: 1642 Phone Number 308-6410 Serial Number: 09/652493
Mail Box and Bldg/Room Location: _____ Results Format Preferred (circle): PAPER DISK E-MAIL
8E12 CM1 9B09

If more than one search is submitted, please prioritize searches in order of need.

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Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: _____

Inventors (please provide full names): _____

_____

Earliest Priority Filing Date: _____

***For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.**

Please search claims 1-8, + 22-24

Please search a method of determining tumor susceptibility by ~~detect~~ detection of α-dystroglycan (see claim 1) in mammalian cells.

Also, for a method of detecting α-dystroglycan in blood serum (see claims 22).

RECEIVED JUL 16 2001

Point of Contact:
Susan Hanley
Technical Info. Specialist
CM1 12C14 Tel: 305-4053

L1

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STAFF USE ONLY	Type of Search		Vendors and cost where applicable	
Searcher: Hanley	NA Sequence (#)		STN	
Searcher Phone #:	AA Sequence (#)		Dialog	
Searcher Location:	Structure (#)		Questel/Orbit	
Date Searcher Picked Up: 8/3	Bibliographic	X	Dr.Link	
Date Completed: 8/6	Litigation		Lexis/Nexis	
Searcher Prep & Review Time:	Fulltext		Sequence Systems	
Clerical Prep Time:	Patent Family		WWW/Internet	
Online Time:	Other		Other (specify)	

BEST AVAILABLE COPY

=> d bib abs hitstr

L26 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2001 ACS
AN 2001:12740 HCAPLUS
DN 134:85131
TI Evaluation of adenocarcinoma of the prostate and breast using anti-**dystroglycan** antibodies
IN Campbell, Kevin P.; Henry, Michael; Cohen, Michael B.
PA University of Iowa Research Foundation, USA
SO PCT Int. Appl., 18 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001001151	A2	20010104	WO 2000-US40206	20000615
WO 2001001151	A3	20010426		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 1999-141149 P 19990625
AB Disclosed is a method for **diagnosing** the **tumorigenic** grade of a malignant tissue. The method entails detg. the amt. of **dystroglycan** protein of the malignant tissue relative to a std. Suitable methods for detg. the amt. of **dystroglycan** protein of the tissue are provided, and include **measuring** the amt. of mRNA transcripts which encode **dystroglycan**, and also performing western blot anal. or immunofluorescence anal. on the tissue components to **detect** <a-**dystroglycan** or <b-**dystroglycan**. An antibody probe which binds specifically to the C-terminus of <b-**dystroglycan**, is provided. This method is applicable to human malignant tissue, esp. adenocarcinoma, and preferably prostate or mammary adenocarcinoma. This method can also be applied to the **detection** of a cancerous disease state in a tissue of a patient, with a decreased level of **dystroglycan** protein being indicative of the presence of cancer. Also disclosed is a method for detg. the prognosis of a patient afflicted with a malignancy by detg. the expression level of the **dystroglycan** gene in a tissue sample of the malignancy, and comparing the expression level to a std., with a decreased level of **dystroglycan** expression being indicative of unfavorable prognosis. A method for identifying an individual at risk for the development of cancer, or an individual at risk for the recurrence of cancer after treatment, is also disclosed. Similarly, a method for identifying individuals at risk for developing cancer by screening for mutations in the **dystroglycan** genes of the individual is also provided. One such mutation is the allelic loss of human chromosome 3p21.

=> d ind

L26 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2001 ACS
IC ICM G01N033-68
CC 15-3 (Immunochemistry)
Section cross-reference(s): 3, 9, 14
ST antibody **dystroglycan** gene mutation adenocarcinoma; prostate mammary adenocarcinoma **immunoassay** Northern blot
IT **Carcinoma**
Mammary gland
Prostate gland
(adenocarcinoma; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT **Diagnosis**
(cancer, **tumorigenic** grade; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT Neoplasm
(**diagnosis, tumorigenic** grade; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT **Glycoproteins, specific or class**
RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(**dystroglycan**; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT Mutation
Northern blot hybridization
Prognosis
Standard substances, analytical
Susceptibility (genetic)
(evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT RNA
RL: AMX (Analytical matrix); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT mRNA
RL: ANT (Analyte); BOC (Biological occurrence); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT **Antibodies**
RL: ARG (Analytical reagent use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT **Immunoassay**
(fluorescence; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT Gene, animal
RL: ANT (Analyte); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(for **dystroglycan**; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT Chromosome
(human 3, 3p21; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT **Immunoassay**
(immunoblotting; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT Alleles
(loss; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)
IT **Glycoproteins, specific or class**
RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL

(Biological study); OCCU (Occurrence); USES (Uses)
(.alpha.-**dystroglycans**; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)

IT **Glycoproteins, specific or class**
RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(.beta.-**dystroglycans**; evaluation of prostate and breast adenocarcinoma using anti-**dystroglycan** antibodies)

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=> d his

     (FILE 'HOME' ENTERED AT 11:39:25 ON 06 AUG 2001)

     FILE 'HCAPLUS' ENTERED AT 11:39:34 ON 06 AUG 2001
L1          199 S BISSELL M?/AU
L2           12 S MUSCHLER J?/AU
L3            3 S L1 AND L2
L4          341 S ?DYSTROGLYCAN?
L5          208 S L1-2
L6            0 S L4 AND L5
L7            8 S L5 AND ?GLYCAN?
L8           26 S L5 AND ASSAY?
L9            9 S L8 AND ?TUMOR?
L10           1 S L8 AND PROTEOLYS?
L11           9 S L9 OR L10
L12           1 S L8 AND ?GLYCAN?
L13          10 S L11-12
                SELECT RN L13 1-10

     FILE 'REGISTRY' ENTERED AT 11:44:25 ON 06 AUG 2001
L14          10 S E1-10

     FILE 'HCAPLUS' ENTERED AT 11:44:34 ON 06 AUG 2001
L15           4 S L13 AND L14
L16           6 S L13 NOT L15
                E GLYCOPROTEIN/CT
                E GLYCOPROTEINS/CT
                E E3+ALL/CT
                E GLYCOPROTEINS/CT
L17       61135 S E3-5
                E DYSTROGLYCANS/CT
                E DYSTROGLYCAN/CT
                E IMMUNOASSAYS/CT
                E IMMUNOASSAY/CT
                E E3+ALL/CT
L18       30943 S E2-3
                E ANTIBODIES/CT
L19      111877 S E3
L20         341 S ?DYSTROGLYCAN?

     FILE 'REGISTRY' ENTERED AT 12:25:35 ON 06 AUG 2001
                E DYSTROGLYCACNN
                E DYSTROGLYCAN/CN

     FILE 'HCAPLUS' ENTERED AT 12:26:30 ON 06 AUG 2001
L21       61304 S L17 OR L20
L22         881 S L21 AND L18
L23         534 S L22 AND L19
L24         521 S L23 AND (?ASSAY? OR DIAGNOS? OR DETECT? OR MEASUR?)
L25          73 S L24 AND (?TUMOR? OR CARCINOMA)
L26           1 S L25 AND L20
L27          73 S L20 AND (ANTIBOD? OR L19)
L28         140 S L20 AND (ANALYT? OR ?ASSAY? OR DIAGNOS? OR DETECT? OR DETERMI
L29          38 S L27 AND L28
L30      430607 S ?TUMOR? OR ?CANCER? OR ?CARCINOM?
L31           9 S L20 AND L30
L32     1139409 S BLOOD OR SERUM OR SERA OR EPITHELIAL?
L33          38 S L20 AND L32
L34           2 S L29 AND L31
L35           7 S L29 AND L33
L36           9 S L34 OR L35
L37           8 S L36 NOT L26
L38         289 S DG AND (ANTIBOD? OR L19)
L39        3244 S DG AND  (ANALYT? OR ?ASSAY? OR DIAGNOS? OR DETECT? OR DETERM?
L40         606 S DG AND ( ?TUMOR? OR ?CANCER? OR ?CARCINOM?)
L41         619 S DG AND L32
L42          20 S L38-41 AND ?GLYCAN?
L43          19 S L42 NOT L36
L44           2 S L29 AND L30
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INVENTOR SEARCH

4 cites

6 cites

using CA indexing

no entries in Reg File

1 cite

8 cites

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L45           0 S L44 NOT L36
L46           9 S L43 AND ANTIBOD?
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9 cites

FILE 'MEDLINE, BIOSIS, SCISEARCH, USPATFULL, WPIDS' ENTERED AT 12:44:27 ON 06 AUG 2001 ← multi file

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L47        1228 S ?DYSTROGLYCAN?
L48    16562102 S ANALY? OR ?ASSAY? OR DIAGNOS? OR DETECT? OR MEASUR? OR DETERM
L49         627 S L47 AND L48
L50          21 S L49 AND (?TUMOR? OR ?CANCER? OR ?CARCINOM?)
L51         361 S L49 AND (MONOCLON? OR ANTIBOD? OR LAMININ)
L52          16 S L50 AND L51
L53          14 DUP REM L52 (2 DUPLICATES REMOVED)
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14 cites

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L54           5 S L50 NOT L52
L55           3 DUP REM L54 (2 DUPLICATES REMOVED)
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3 cites

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L56          31 S L47  AND (?TUMOR? OR ?CANCER? OR ?CARCINOM?)
L57          10 S L56 NOT (L52 OR L54)
L58           9 DUP REM L57 (1 DUPLICATE REMOVED)
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9 cites - looking for 103's of DG w/ tumor cells.

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L59         119 S L47(5A)L48
L60          79 S L59 AND (MONOCLON? OR ANTIBOD? OR LAMININ)
L61          35 S L59 AND (TISSUE OR EPITHEL? OR BLOOD OR SERUM)
L62          85 S L59 AND CELL
L63          20 DUP REM L61 (15 DUPLICATES REMOVED)
L64          19 S L63 NOT (L52 OR L54 OR L57)
L65          41 DUP REM L62 (44 DUPLICATES REMOVED)
L66          39 S L65 NOT (L52 OR L54 OR L57)
L67          45 S L64 OR L66
L68          19 S L67 AND (MONOCLON? OR ANTIBOD? OR IMMUNOASS?)
L69          12 S L67 AND FRAGMENT
L70          22 S L68-69
```

22 cites ← looking for 103's for DG w/ antibodies/assay

=> d bib abs hitstr 1

L15 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:215367 HCAPLUS
DN 131:17229
TI .alpha.1 and .alpha.2 integrins mediate invasive activity of mouse mammary carcinoma cells through regulation of stromelysin-1 expression
AU Lochter, Andre; Navre, Marc; Werb, Zena; **Bissell, Mina J.**
CS Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, 94720, USA
SO Mol. Biol. Cell (1999), 10(2), 271-282
CODEN: MBCEEV; ISSN: 1059-1524
PB American Society for Cell Biology
DT Journal
LA English
AB **Tumor** cell invasion relies on cell migration and extracellular matrix **proteolysis**. We investigated the contribution of different integrins to the invasive activity of mouse mammary carcinoma cells. Antibodies against integrin subunits .alpha.6 and .beta.1, but not against .alpha.1 and .alpha.2, inhibited cell locomotion on a reconstituted basement membrane in two-dimensional cell migration **assays**, whereas antibodies against .beta.1, but not against .alpha.6 or .alpha.2, interfered with cell adhesion to basement membrane constituents. Blocking antibodies against .alpha.1 integrins impaired only cell adhesion to type IV collagen. Antibodies against .alpha.1, .alpha.2, .alpha.6, and .beta.1, but not .alpha.5, integrin subunits reduced invasion of a reconstituted basement membrane. Integrins .alpha.1 and .alpha.2, which contributed only marginally to motility and adhesion, regulated proteinase prodn. Antibodies against .alpha.1 and .alpha.2, but not .alpha.6 and .beta.1, integrin subunits inhibited both transcription and protein expression of the matrix metalloproteinase stromelysin-1. Inhibition of **tumor** cell invasion by antibodies against .alpha.1 and .alpha.2 was reversed by addn. of recombinant stromelysin-1. In contrast, stromelysin-1 could not rescue invasion inhibited by anti-.alpha.6 antibodies. Our data indicate that .alpha.1 and .alpha.2 integrins confer invasive behavior by regulating stromelysin-1 expression, whereas .alpha.6 integrins regulate cell motility. These results provide new insights into the specific functions of integrins during **tumor** cell invasion.
IT **81669-70-7**, Metalloproteinase
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(80,000-mol.-wt.; .alpha.1 and .alpha.2 integrins confer invasive behavior by regulating stromelysin-1 expression and .alpha.6 integrins regulate cell motility in mammary carcinoma)
RN 81669-70-7 HCAPLUS
CN Proteinase, metallo- (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
IT **9001-92-7**, Proteinase **79955-99-0**, Stromelysin-1
RL: ADV (Adverse effect, including toxicity); BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(.alpha.1 and .alpha.2 integrins confer invasive behavior by regulating stromelysin-1 expression and .alpha.6 integrins regulate cell motility in mammary carcinoma)
RN 9001-92-7 HCAPLUS
CN Proteinase (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
RN 79955-99-0 HCAPLUS
CN Stromelysin 1 (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
IT **146480-35-5**, Gelatinase A **146480-36-6**, Gelatinase B
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(.alpha.1 and .alpha.2 integrins confer invasive behavior by regulating stromelysin-1 expression and .alpha.6 integrins regulate cell motility in mammary carcinoma)

RN 146480-35-5 HCAPLUS
CN Gelatinase A (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
RN 146480-36-6 HCAPLUS
CN Gelatinase B (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
RE.CNT 65
RE .
(1) Brooks, P; Cell 1996, V85, P683 HCAPLUS
(2) Cannistra, S; Gynecol Oncol 1995, V58, P216 HCAPLUS
(3) Chao, C; Cancer Res 1996, V56, P4811 HCAPLUS
(4) Chintala, S; Cancer Lett 1996, V103, P201 HCAPLUS
(5) Cress, A; Cancer Metastasis Rev 1995, V14, P219 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 2

L15 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:805579 HCAPLUS
DN 130:120000
TI Reciprocal interactions between .beta.1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology
AU Wang, Fei; Weaver, Valerie M.; Petersen, Ole W.; Larabell, Carolyn A.; Dedhar, Shoukat; Briand, Per; Lupu, Ruth; **Bissell, Mina J.**
CS Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1998), 95(25), 14821-14826
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB Anchorage and growth factor independence are cardinal features of the transformed phenotype. Although it is logical that the two pathways must be coregulated in normal tissues to maintain homeostasis, this has not been demonstrated directly. We showed previously that down-modulation of .beta.1-integrin signaling reverted the malignant behavior of a human breast **tumor** cell line (T4-2) derived from phenotypically normal cells (HMT-3522) and led to growth arrest in a three-dimensional (3D) basement membrane **assay** in which the cells formed tissue-like acini. Here, we show that there is a bidirectional cross-modulation of .beta.1-integrin and epidermal growth factor receptor (EGFR) signaling via the mitogen-activated protein kinase (MAPK) pathway. The reciprocal modulation does not occur in monolayer (2D) cultures. Antibody-mediated inhibition of either of these receptors in the **tumor** cells, or inhibition of MAPK kinase, induced a concomitant down-regulation of both receptors, followed by growth-arrest and restoration of normal breast tissue morphogenesis. Cross-modulation and tissue morphogenesis were assocd. with attenuation of EGF-induced transient MAPK activation. To specifically test EGFR and .beta.1-integrin interdependency, EGFR was overexpressed in nonmalignant cells, leading to disruption of morphogenesis and a compensatory up-regulation of .beta.1-integrin expression, again only in 3D. Our results indicate that when breast cells are spatially organized as a result of contact with basement membrane, the signaling pathways become coupled and bidirectional. They further explain why breast cells fail to differentiate in monolayer cultures in which these events are mostly uncoupled. Moreover, in a subset of **tumor** cells in which these pathways are misregulated but functional, the cells could be "normalized" by manipulating either pathway.
IT **142243-02-5**, Mitogen-activated protein kinase **142805-58-1**
, MAP kinase kinase
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.beta.1-integrin and EGF receptor reciprocal interactions in three-dimensional basement membrane breast cultures)
RN 142243-02-5 HCAPLUS
CN Kinase (phosphorylating), mitogen-activated protein (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
RN 142805-58-1 HCAPLUS
CN Kinase (phosphorylating), mitogen-activated protein kinase (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
IT **62229-50-9**, Epidermal growth factor
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(.beta.1-integrin and EGF receptor reciprocal interactions in three-dimensional basement membrane breast cultures)
RN 62229-50-9 HCAPLUS
CN Epidermal growth factor (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
RE.CNT 41
RE
(2) Boudreau, N; Trends Cell Biol 1995, V5, P1 HCAPLUS

(3) Briand, P; Cancer Res 1996, V56, P2039 HCAPLUS
(5) Chen, Q; J Biol Chem 1994, V269, P26602 HCAPLUS
(6) Clark, E; J Biol Chem 1996, V271, P14814 HCAPLUS
(7) Clark, E; Science 1995, V268, P233 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs hitstr 3

L15 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2001 ACS
AN 1993:140978 HCAPLUS
DN 118:140978
TI A novel transcriptional enhancer is involved in the prolactin- and extracellular matrix-dependent regulation of .beta.-casein gene expression
AU Schmidhauser, Christian; Casperson, Gerald F.; Myers, Connie A.; Sanzo, Kimberly T.; Bolten, Suzanne; **Bissell, Mina J.**
CS Lawrence Berkeley Lab., Univ. California, Berkeley, CA, 94720, USA
SO Mol. Biol. Cell (1992), 3(6), 699-709
CODEN: MBCEEV; ISSN: 1059-1524
DT Journal
LA English
AB Lactogenic hormones and extracellular matrix (ECM) act synergistically to regulate .beta.-casein expression in culture. A functional subpopulation of the mouse mammary epithelial cell strain COMMA-1D (designated CID 9) was developed which expresses high levels of .beta.-casein, forms alveolar-like structures when plated onto the EHS **tumor**-derived matrix, and secretes .beta.-casein unidirectionally into a lumen. It was shown that ECM- and prolactin-dependent regulations of .beta.-casein occur mainly at the transcriptional level and that 5' sequences play an important role in these regulations. To address the question of the nature of the DNA sequence requirements for such regulation, the bovine .beta.-casein gene promoter in these cells was analyzed. A 160-bp transcriptional enhancer (BCE1) was located within the 5' flanking region of the .beta.-casein gene. Using functional **assays**, BCE1 was shown to contain responsive elements for prolactin- and ECM-dependent regulation. BCE1 placed upstream of a truncated and inactive .beta.-casein promoter (the shortest extending from -89 to +42 bp with regard to the transcription start site) reconstitutes a promoter even more potent than the intact promoter, which contains BCE1 in its normal context more than 1.5 kb upstream. This small fusion promoter also reconstitutes the normal pattern of regulation, including a requirement for both prolactin and ECM and a synergistic action of prolactin and hydrocortisone. By replacing the milk promoter with a heterologous viral promoter, it was demonstrated that BCE1 participates in the prolactin- and ECM-mediated regulation.
IT **146482-53-3**
RL: PRP (Properties); BIOL (Biological study)
(nucleotide sequence of)
RN 146482-53-3 HCAPLUS
CN DNA (cattle clone b.beta.cas-1790+42/CAT .beta.-casein gene enhancer BCE1 region-containing fragment) (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
IT **9002-62-4**, Prolactin, biological studies
RL: BIOL (Biological study)
(.beta.-casein gene of mouse CID9 cells regulated by, enhancer BCE1 mediation of)
RN 9002-62-4 HCAPLUS
CN Prolactin (8CI, 9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

=> d bib abs hitstr 4

L15 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2001 ACS
AN 1987:595597 HCAPLUS
DN 107:195597
TI Casein gene expression in mouse mammary epithelial cell lines: dependence upon extracellular matrix and cell type
AU Medina, Daniel; Li, M. L.; Oborn, C. J.; **Bissell, M. J.**
CS Dep. Cell Biol., Baylor Coll. Med., Houston, TX, 77030, USA
SO Exp. Cell Res. (1987), 172(1), 192-203
CODEN: ECREAL; ISSN: 0014-4827
DT Journal
LA English
AB The COMMA-D mammary cell line exhibits mammary-specific functional differentiation under appropriate conditions in cell culture. The cytol. heterogeneous COMMA-D parental line and the clonal lines DB-1, TA-5, and FA-1 derived from the COMMA-D parent were examd. for similar properties of functional differentiation. In monolayer cell culture, the cell lines DB-1, TA-5, FA-1, and MA-4 were examd. for expression of mammary-specific and epithelial-specific proteins by an indirect immunofluorescence **assay**. The clonal cell lines were relatively homogeneous in their resp. staining properties and seemed to represent 3 subpopulations found in the heterogeneous parental COMMA-D line. None of the 4 clonal lines appeared to represent myoepithelial cells. The cell lines were examd. for expression of .beta.-casein mRNA in the presence or absence of prolactin. The heterogeneous COMMA-D line, but none of the clonal lines, was induced by the presence of prolactin to produce significantly increased levels of .beta.-casein mRNA. The inducibility of .beta.-casein in the COMMA-D cell line was further enhanced by a reconstituted basement membrane prepn. enriched in laminin, collagen IV, and **proteoglycans**. Individual matrix components of laminin, fibronectin, heparan sulfate, heparan, or hyaluronic acid were not effective as substrata for the induction of .beta.-casein mRNA. Thus the functional response of inducible mammary cell populations is evidently a result of interaction among hormones, multiple extracellular matrix components, and specific cell types.
IT **9002-62-4**, Prolactin, biological studies
RL: BIOL (Biological study)
(.beta.-casein-specifying mRNA of mammary gland epithelium in response to, extracellular matrix in relation to)
RN 9002-62-4 HCAPLUS
CN Prolactin (8CI, 9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

=> d bib abs l16 1

L16 ANSWER 1 OF 6 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:41808 HCAPLUS
DN 132:164446
TI E7-transduced human breast epithelial cells show partial differentiation in three-dimensional culture
AU Spancake, Kimberly M.; Anderson, Christine B.; Weaver, Valerie M.; Matsunami, Norisada; **Bissell, Mina J.**; White, Raymond L.
CS Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, 84112, USA
SO Cancer Res. (1999), 59(24), 6042-6045
CODEN: CNREA8; ISSN: 0008-5472
PB AACR Subscription Office
DT Journal
LA English
AB Disruption of the retinoblastoma (RB) **tumor** suppressor pathway is a common and important event in breast carcinogenesis. To examine the role of the retinoblastoma protein (pRB) in this process, the authors created human mammary epithelial cells (HMEC) deficient for pRB by infecting primary outgrowth from breast organoids with the human papillomavirus type 16 (HPV16) E7 gene. HPV16 E7 binds to and inactivates pRB and also causes a significant down-regulation of the protein. Culturing normal HMEC in a reconstituted basement membrane (rBM) provides a correct environment and signaling cues for the formation of differentiated, acini-like structures. When cultured in this rBM, HMEC+E7 were found to respond morphol. as normal HMEC and form acinar structures. In contrast to normal HMEC, many of the cells within the HMEC+E7 structures were not growth arrested, as detd. by a 5-bromo-2'-deoxyuridine incorporation **assay**. PRB deficiency did not affect polarization of these structures, as indicated by the normal localization of the cell-cell adhesion marker E-cadherin and the basal deposition of a collagen IV membrane. However, in HMEC+E7 acini, the authors were unable to detect by immunofluorescence microscopy the milk protein lactoferrin or cytokeratin 19, both markers of differentiation expressed in the normal HMEC structures. These data suggest that loss of RB in vivo would compromise differentiation, predisposing these cells to future **tumor**-promoting actions.
RE.CNT 26
RE
(1) Berezutskaya, E; Cell Growth Differ 1997, V8, P1277 HCAPLUS
(2) Bissell, M; Cancer Res 1999, V59, P1757s HCAPLUS
(3) Boyer, S; Cancer Res 1996, V56, P4620 HCAPLUS
(5) Close, M; J Cell Sci 1997, V110, P2861 HCAPLUS
(8) Gu, W; Cell 1993, V72, P309 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

```
=> d ind 1

L16  ANSWER 1 OF 6  HCAPLUS  COPYRIGHT 2001 ACS
CC   14-1 (Mammalian Pathological Biochemistry)
     Section cross-reference(s): 10
ST   papillomavirus E7 protein breast epithelium differentiation
IT   Keratins
     RL: BOC (Biological occurrence); BIOL (Biological study); OCCU
     (Occurrence)
        (19; papillomavirus E7-mediated deficiency in retinoblastoma protein in
        breast epithelial cells impairs differentiation-assocd. expression of)
IT   Transcription factors
     RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
        (E7; papillomavirus E7-mediated deficiency in retinoblastoma protein in
        breast epithelial cells impairs cell cycle regulation and
        differentiation)
IT   Transcription factors
     RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
        (Rb; papillomavirus E7-mediated deficiency in retinoblastoma protein in
        breast epithelial cells impairs cell cycle regulation and
        differentiation)
IT   Mammary gland
        (epithelium; papillomavirus E7-mediated deficiency in retinoblastoma
        protein in breast epithelial cells impairs cell cycle regulation and
        differentiation)
IT   Mammary gland
        (neoplasm; papillomavirus E7-mediated deficiency in retinoblastoma
        protein in breast epithelial cells impairs cell cycle regulation and
        differentiation in relation to progression to)
IT   Cell cycle
     Cell differentiation
     Human papillomavirus 16
        (papillomavirus E7-mediated deficiency in retinoblastoma protein in
        breast epithelial cells impairs cell cycle regulation and
        differentiation)
IT   Transformation, neoplastic
        (papillomavirus E7-mediated deficiency in retinoblastoma protein in
        breast epithelial cells impairs cell cycle regulation and
        differentiation in relation to)
IT   Lactoferrins
     RL: BOC (Biological occurrence); BIOL (Biological study); OCCU
     (Occurrence)
        (papillomavirus E7-mediated deficiency in retinoblastoma protein in
        breast epithelial cells impairs differentiation-assocd. expression of)
```

=> d bib abs l16 2

L16 ANSWER 2 OF 6 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:241818 HCAPLUS
DN 131:42712
TI Tissue structure, nuclear organization, and gene expression in normal and malignant breast
AU **Bissell, Mina J.**; Weaver, Valerie M.; Lelievre, Sophie A.; Wang, Fei; Petersen, Ole W.; Schmeichel, Karen L.
CS Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA
SO Cancer Res. (1999), 59(7, Suppl.), 1757S-1764S
CODEN: CNREA8; ISSN: 0008-5472
PB AACR Subscription Office
DT Journal; General Review
LA English
AB A review, with 61 refs. Because every cell within the body has the same genetic information, a significant problem in biol. is to understand how cells within a tissue express genes selectively. A sophisticated network of phys. and biochem. signals converge in a highly orchestrated manner to bring about the exquisite regulation that governs gene expression in diverse tissues. Thus, the ultimate decision of a cell to proliferate, express tissue-specific genes, or apoptose must be a coordinated response to its adhesive, growth factor, and hormonal milieu. The unifying hypothesis examd. in this overview is that the unit of function in higher organisms is neither the genome nor the cell alone but the complex, three-dimensional tissue. This is because there are bidirectional connections between the components of the cellular microenvironment (growth factors, hormones, and extracellular matrix) and the nucleus. These connections are made via membrane-bound receptors and transmitted to the nucleus, where the signals result in modifications to the nuclear matrix and chromatin structure and lead to selective gene expression. Thus, cells need to be studied "in context", i.e., within a proper tissue structure, if one is to understand the bidirectional pathways that connect the cellular microenvironment and the genome. In the last decades, we have used well-characterized human and mouse mammary cell lines in "designer microenvironments" to create an appropriate context to study tissue-specific gene expression. The use of a three-dimensional culture **assay**, developed with reconstituted basement membrane, has allowed us to distinguish normal and malignant human breast cells easily and rapidly. Whereas normal cells become growth arrested and form organized "acini," **tumor** cells continue to grow, pile up, and in general fail to respond to extracellular matrix and microenvironmental cues. By correcting the extracellular matrix-receptor (integrin) signaling and balance, we have been able to revert the malignant phenotype when a human breast **tumor** cell is cultured in, or on, a basement membrane. Most recently, we have shown that whereas .beta.1 integrin and epidermal growth factor receptor signal transduction pathways are integrated reciprocally in three-dimensional cultures, on tissue culture plastic (two-dimensional monolayers), these are not coordinated. Finally, we have demonstrated that, rather than passively reflecting changes in gene expression, nuclear organization itself can modulate cellular and tissue phenotype. We conclude that the structure of the tissue is dominant over the genome, and that we may need a new paradigm for how epithelial-specific genes are regulated in vivo. We also argue that unless the structure of the tissue is critically altered, malignancy will not progress, even in the presence of multiple chromosomal mutations.
RE.CNT 61
RE
(2) Alroy, I; FEBS Lett 1997, V410, P83 HCAPLUS
(4) Bissell, M; Int Rev Cytol 1981, V70, P27 HCAPLUS
(5) Bissell, M; J Theor Biol 1982, V99, P31 HCAPLUS
(6) Bode, J; Crit Rev Eukaryotic Gene Expression 1996, V6, P115 HCAPLUS
(7) Boudreau, N; Proc Natl Acad Sci USA 1996, V93, P3509 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

```
=> d ind 2

L16  ANSWER 2 OF 6  HCAPLUS  COPYRIGHT 2001 ACS
CC   14-0 (Mammalian Pathological Biochemistry)
     Section cross-reference(s): 3
ST   review breast cancer tissue structure gene expression
IT   Gene
        (expression; tissue structure, nuclear organization, and gene
        expression in normal and malignant breast)
IT   Mammary gland
        (neoplasm; tissue structure, nuclear organization, and gene expression
        in normal and malignant breast)
IT   Cell nucleus
     Mammary gland
     Signal transduction, biological
        (tissue structure, nuclear organization, and gene expression in normal
        and malignant breast)
IT   Epidermal growth factor receptors
     RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
        (tissue structure, nuclear organization, and gene expression in normal
        and malignant breast)
IT   Integrins
     RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
        (.beta.1; tissue structure, nuclear organization, and gene expression
        in normal and malignant breast)
```

=> d bib abs l16 3

L16 ANSWER 3 OF 6 HCAPLUS COPYRIGHT 2001 ACS
AN 1997:345803 HCAPLUS
DN 127:63732
TI The importance of the microenvironment in breast cancer progression: recapitulation of mammary **tumorigenesis** using a unique human mammary epithelial cell model and a three-dimensional culture **assay**
AU Weaver, V.M.; Fischer, A.H.; Peterson, O.W.; **Bissell, M.J.**
CS Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA
SO Biochem. Cell Biol. (1996), 74(6), 833-851
CODEN: BCBIEQ; ISSN: 0829-8211
PB National Research Council of Canada
DT Journal; General Review
LA English
AB A review with .apprx.160 refs. The extracellular matrix (ECM) is a dominant regulator of tissue development and homeostasis. "Designer microenvironments" in culture and in vivo model systems have shown that the ECM regulates growth, differentiation, and apoptosis in murine and human mammary epithelial cells (MEC) through a hierarchy of transcriptional events involving the intricate interplay between sol. and phys. signaling pathways. Furthermore, these studies have shown that these pathways direct and in turn are influenced by the tissue structure. Tissue structure is directed by the cooperative interactions of the cell-cell and cell-ECM pathways and can be modified by stromal factors. Not surprisingly then, loss of tissue structure and alterations in ECM components are assocd. with the appearance and dissemination of breast **tumors**, and malignancy is assocd. with perturbations in cell adhesion, changes in adhesion mols., and a stromal reaction. Several lines of evidence now support the contention that the pathogenesis of breast cancer is detd. (at least in part) by the dynamic interplay between the ductal epithelial cells, the microenvironment, and the tissue structure (acini). Thus, to understand the mechanisms involved in carcinogenesis, the role of the microenvironment (ECM as well as the stromal cells) with respect to tissue structure should be considered and studied. Towards this goal, the authors have established a unique human MEC model of **tumorigenesis**, which in concert with a three-dimensional **assay**, recapitulates many of the genetic and morphol. changes obsd. in breast cancer in vivo. The authors are currently using this system to understand the role of the microenvironment and tissue structure in breast cancer progression.

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=> d ind 3

L16  ANSWER 3 OF 6  HCAPLUS  COPYRIGHT 2001 ACS
CC   14-0 (Mammalian Pathological Biochemistry)
     Section cross-reference(s): 9
ST   review breast cancer microenvironment adhesion mol; culture assay
     breast cancer microenvironment review
IT   Breast tumors
     Disease models
     Extracellular matrix
     Mammalian tissue culture
     Signal transduction (biological)
        (microenvironment in breast cancer progression studied by human mammary
        epithelial cell model and three-dimensional culture assay in
        relation to cell adhesion mols.)
IT   Cell adhesion molecules
     RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
        (microenvironment in breast cancer progression studied by human mammary
        epithelial cell model and three-dimensional culture assay in
        relation to cell adhesion mols.)
```

=> d bib abs l16 4

L16 ANSWER 4 OF 6 HCAPLUS COPYRIGHT 2001 ACS
AN 1995:756888 HCAPLUS
DN 123:253057
TI The development of a functionally relevant cell culture model of progressive human breast cancer
AU Weaver, Valerie M.; Howlett, Anthony R.; Langton-Webster, Beatrice; Petersen, Ole W.; **Bissell, Mina J.**
CS Lawrence Berkeley Laboratory, University of California, Berkeley, CA, 94720, USA
SO Semin. Cancer Biol. (1995), Volume Date 1995, 6(3), 175-84
CODEN: SECBE7; ISSN: 1044-579X
DT Journal
LA English
AB Normal mammary homeostasis, and by implication **tumorigenesis**, are dependent upon the dynamic interplay between epithelial cells, stromal components, and the extracellular matrix. To study the evolution of human breast cancer, a functionally relevant cell culture model is required which recognizes the complexity of the mammary gland's microenvironment. The development of an appropriate breast epithelial cancer cell model will be dependent on the ability to recreate the normal and neoplastic tissue microenvironment in culture. Towards this goal, a 3-dimensional extracellular matrix (ECM) **assay**, employing a reconstituted basement membrane, has been developed which allows for the rapid and accurate discrimination of normal and neoplastic cells when cultured. To investigate stromal/epithelial cell interactions, the authors have developed a **tumor** environment **assay** which essentially mirrors the **tumor** microenvironment histol. The use of a novel, near diploid, human breast epithelial cell line, HMT-3522, which has transformed spontaneously with passage in culture, together with these 3-dimensional culture **assays**, is expected to provide meaningful markers of initiation and progression.

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=> d ind 4

L16  ANSWER 4 OF 6  HCAPLUS  COPYRIGHT 2001 ACS
CC   14-1 (Mammalian Pathological Biochemistry)
ST   human mammary cancer cell culture model
IT   Animal cell line
        (HMT-3522; development of functionally relevant cell culture model of
        progressive human breast cancer)
IT   Extracellular matrix
        (development of functionally relevant cell culture model of progressive
        human breast cancer)
IT   Mammary gland
        (neoplasm, development of functionally relevant cell culture model of
        progressive human breast cancer)
```

=> d bib abs l16 5

L16 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2001 ACS
AN 1995:567651 HCAPLUS
DN 123:6576
TI Cellular growth and survival are mediated by .beta.1 integrins in normal human breast epithelium but not in breast carcinoma
AU Howlett, Anthony R.; Bailey, Nina; Bamsky, Caroline; Petersen, Ole W.; **Bissell, Mina J.**
CS Lawrence Berkeley Laboratory, University of California, Berkeley, CA, 94720, USA
SO J. Cell Sci. (1995), 108(5), 1945-57
CODEN: JNCSAI; ISSN: 0021-9533
DT Journal
LA English
AB We previously established a rapid three-dimensional **assay** for discrimination of normal and malignant human breast epithelial cells using a laminin-rich reconstituted basement membrane. In this **assay**, normal epithelial cells differentiate into well-organized acinar structures whereas **tumor** cells fail to recapitulate this process and produce large, disordered colonies. The data suggest that breast acinar morphogenesis and differentiation is regulated by cell-extra-cellular matrix (ECM) interactions and that these interactions are altered in malignancy. Here, we investigated the role of ECM receptors (integrins) in these processes and report on the expression and function of potential laminin receptor in normal and **tumorigenic** breast epithelial cells. Immunocytochem. anal. showed that normal and carcinoma cells in a three-dimensional substratum express profiles of integrins similar to normal and malignant breast tissues in situ. Normal cells express .alpha.1, .alpha.2, .alpha.3, .alpha.6, .beta.1 and .beta.4 integrin subunits, whereas breast carcinoma cells show variable losses, disordered expression, or downregulation of these subunits. Function-blocking expts. using inhibitory antiintegrin subunit antibodies showed a >5-fold inhibition of the formation of acinar structures by normal cells in the presence of either anti-.beta.1 or anti-.alpha.3 antibodies, whereas anti-.alpha.2 or -.alpha.6 had little or no effect. In expts. where collagen type I gels were used instead of basement membrane, acinar morphogenesis was blocked by anti-.beta.1 and -.alpha.2 antibodies but not by anti=.alpha.3. These data suggest a specificity of integrin utilization dependent on the ECM ligands encountered by the cell. The interruption of normal acinar morphogenesis by anti-integrin antibodies was assocd. with an inhibition of cell growth and induction of apoptosis. Function-blocking antibodies had no inhibitory effect on the rate of **tumor** cell growth, survival or capacity to form colonies. Thus under our culture conditions breast acinar formation is at least a two-step process involving .beta.1-integrin-dependent cellular growth followed by polarization of the cells into organized structures. The regulation of this pathway appears to be impaired or lost in the **tumor** cells, suggesting that **tumor** colony formation occurs by independent mechanisms and that loss of proper integrin-mediated cell-ECM interaction may be crit. to breast **tumor** formation.

=> d bib abs l16 6

L16 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2001 ACS
AN 1995:349831 HCAPLUS
TI The origin of myofibroblasts in breast cancer: recapitulation of **tumor** environment in culture unravels diversity and implicates converted fibroblasts and recruited smooth muscle cells
AU Roennov-Jessen, Lone; Petersen, Ole W.; Koteliansky, Victor E.; **Bissell, Mina J.**
CS Struct. Cell Biol. Unit, Inst. Med. Anat., Copenhagen, DK-2200, Den.
SO J. Clin. Invest. (1995), 95(2), 859-73
CODEN: JCINAO; ISSN: 0021-9738
DT Journal
LA English
AB The origin of myofibroblasts in stromal reaction has been a subject of controversy. To address this question definitively, we developed techniques for purifn. and characterization of major stromal cell types. We defined a panel of markers that could, in combination, unequivocally distinguish these cells types by immunocytochem., iso-elec. focusing, immunoblotting, and two-dimensional gel electrophoresis. We than devised an **assay** to recapitulate in culture, within two weeks of incubation, crit. aspectes of the icroenvironment in vivo including the typical tissue histol. and stromal reaction When confronted with **tumor** cells in this **assay**, fibroblasts readily converted into a graded pattern of myogenic differentiation, strongest in the immediate vicinity of **tumor** cells. Vascular smooth muscle cells (VSMC), in contrast, did not change appreciably and remained coordinately smoth muscle differentiated. Midcapillary pericytes showed only a slight propensity for myogenic differentiation. Anal. of ten primary **tumors** implicated converted fibroblasts (10/10), vascular smooth muscle cells (4/10), and pericytes (1/10) in the stromal reaction **Tumor** cells were shown to specifically denude the venules both in culture and in vivo, explaining the VSMC phenotype in the stroma. The establishment of this **assay** and clarification of the origin of these cells pave the way for further anal. of the mechanisms of conversion, and the consequence of such heterogeneity for diagnosis and treatment.

=> d ind 6

L16 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2001 ACS

=> d bib abs 1

L37 ANSWER 1 OF 8 HCAPLUS COPYRIGHT 2001 ACS
AN 2001:370108 HCAPLUS
TI **Dystroglycan** distribution in adult mouse brain: a light and electron microscopy study
AU Zaccaria, M. L.; Di Tommaso, F.; Brancaccio, A.; Paggi, P.; Petrucci, T. C.
CS Dipartimento di Biologia Cellulare e dello Sviluppo, Universita "La Sapienza", Rome, 00185, Italy
SO Neuroscience (Oxford, U. K.) (2001), 104(2), 311-324
CODEN: NRSCDN; ISSN: 0306-4522
PB Elsevier Science Ltd.
DT Journal
LA English
AB **Dystroglycan**, originally identified in muscle as a component of the dystrophin-assocd. glycoprotein complex, is a ubiquitously expressed cell-surface receptor that forms a transmembrane link between the extracellular matrix and the cytoskeleton. It contains two subunits, .alpha. and .beta., formed by proteolytic cleavage of a common precursor. In the brain, different neuronal subtypes and glial cells may express **dystroglycan** in complex with distinct cytoplasmic proteins such as dystrophin, utrophin and their truncated forms. To examine the distribution of **dystroglycan** in adult mouse brain, we raised **antibodies** against the recombinant amino- and carboxyl-terminal domains of .alpha.-**dystroglycan**. On western blot, the **antibodies** recognized specifically .alpha.-**dystroglycan** in cerebellar exts. Using light microscopy, .alpha.-**dystroglycan** was found in neurons of the cerebral cortex, hippocampus, olfactory bulb, basal ganglia, thalamus, hypothalamus, brainstem and cerebellum, where dystrophin and its truncated isoforms are also known to be present. Electron microscopy revealed that .alpha.-**dystroglycan** immunoreactivity was preferentially assocd. with the postsynaptic specializations. **Dystroglycan** immunostaining was also **detected** in perivascular astrocytes and in those facing the pia mater, where utrophin and dystrophin truncated isoforms are present. The cell body and endfeet of astrocytes around **blood** vessels and the endothelial cells at the **blood**-brain barrier also expressed **dystroglycan**.From these data, we suggest that **dystroglycan**, by bridging the extracellular matrix and the cytoskeleton, may play an important functional role at specialized intercellular contacts, synapses and the **blood**-brain barrier, whose structural and functional organization strictly depend on the integrity of the extracellular matrix-cytoskeleton linkage.
RE.CNT 58
RE
(1) Ahn, A; Nat Genet 1993, V3, P283 HCAPLUS
(2) Apel, E; Neuron 1995, V15, P115 HCAPLUS
(3) Blake, D; Trends Neurosci 2000, V23, P92 HCAPLUS
(4) Bowe, M; J Cell Biol 2000, V148, P801 HCAPLUS
(5) Bowe, M; Neuron 1994, V12, P1173 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 2

L37 ANSWER 2 OF 8 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:813154 HCAPLUS
DN 134:111892
TI Structural and functional analysis of the recombinant G domain of the laminin .alpha.4 chain and its proteolytic processing in tissues
AU Talts, Jan F.; Sasaki, Takako; Miosge, Nicolai; Gohring, Walter; Mann, Karlheinz; Mayne, Richard; Timpl, Rupert
CS Max-Planck-Institut fur Biochemie, Martinsried, D-82152, Germany
SO J. Biol. Chem. (2000), 275(45), 35192-35199
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB The C-terminal G domains of laminin .alpha. chains have been implicated in various cellular and other interactions. The G domain of the .alpha.4 chain was now produced in transfected mammalian cells as 2 tandem arrays of LG modules, .alpha.4LG1-3 and .alpha.4LG4-5. The recombinant fragments were shown to fold into globular structures and could be distinguished by specific **antibodies**. Both fragments were able to bind to heparin, sulfatides, and the microfibrillar fibulin-1 and fibulin-2. They were, however, poor substrates for cell adhesion and had only a low affinity for the .alpha.-**dystroglycan** receptor when compared with the G domains of the laminin .alpha.1 and .alpha.2 chains. Yet **antibodies** to .alpha.4LG1-3 but not to .alpha.4LG4-5 clearly inhibited .alpha.6.beta.1 integrin-mediated cell adhesion to laminin-8, indicating the participation of .alpha.4LG1-3 in a cell-adhesive structure of higher complexity. Proteolytic processing within a link region between the .alpha.4LG3 and .alpha.4LG4 modules was shown to occur during recombinant prodn. and in endothelial and Schwann cell culture. Cleavage could be attributed to 3 different peptide bonds and is accompanied by the release of the .alpha.4LG4-5 segment. Immunohistol. demonstrated abundant staining of .alpha.4LG1-3 in vessel walls, adipose, and perineural tissue. No significant staining was found for .alpha.4LG4-5, indicating their loss from tissues. Immunogold staining demonstrated an assocn. of the .alpha.4 chain primarily with microfibrillar regions rather than with basement membranes, while laminin .alpha.2 chains appear primarily assocd. with various basement membranes.
RE.CNT 53
RE
(1) Andac, Z; J Mol Biol 1999, V287, P253 HCAPLUS
(3) Aumailley, M; Eur J Biochem 1989, V184, P241 HCAPLUS
(4) Aumailley, M; Exp Cell Res 1989, V181, P463 HCAPLUS
(5) Aumailley, M; Exp Cell Res 1991, V196, P177 HCAPLUS
(6) Aumailley, M; FEBS Lett 1990, V262, P82 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 3

L37 ANSWER 3 OF 8 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:529246 HCAPLUS
DN 131:168353
TI Identification of loci involved in accelerated wound healing and the development of new wound healing promoters
IN Heber-Katz, Ellen
PA The Wistar Institute, USA
SO PCT Int. Appl., 136 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9941364	A2	19990819	WO 1999-US2962	19990212
	WO 9941364	A3	19991223		
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 9926720	A1	19990830	AU 1999-26720	19990212
	EP 1053309	A1	20001122	EP 1999-906924	19990212
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI	US 1998-74737	A2	19980213		
	US 1998-97937	A2	19980826		
	US 1998-102051	A2	19980928		
	WO 1999-US2962	W	19990212		

AB Genes that quant. improve the efficiency and effectiveness of wound healing in mice are identified. Wound healing is **assayed** by measuring the time taken for a 2 mm hole punched into the ear to heal. The genes or gene products may be useful in the development of new wound healing promoters, including agents for treatment of central and peripheral nerve wounds. Wound healing in the rapidly healing mouse line MRL was studied. In comparison to the C57BL/6 line, the MRL mice showed more extensive vascularization around wounds with rapid development of sebaceous glands and hair follicles and the unexpected appearance of adipocytes. These mice also showed improved healing of damage to the optic and sciatic nerve after crushing, and of the spinal cord after complete transection. Using the difference in wound healing behavior of the two lines, genetic polymorphisms assocd. with QTLs affecting wound healing were identified. The accelerated healing of the MRL line was lost with aging, and this appeared to be as a result of T-cell actions. Macrophages from the MRL accelerated wound healing in control mice.

=> d ind 3

L37 ANSWER 3 OF 8 HCAPLUS COPYRIGHT 2001 ACS
IC ICM C12N015-00
ICS C12N005-18; C07K014-47; C07K014-705; C07K016-18; C12Q001-68; A01K067-027
CC 13-6 (Mammalian Biochemistry)
Section cross-reference(s): 3, 15
ST wound healing gene expression; quant genetics wound healing genetic polymorphism
IT Keratins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(1, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Keratins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(14, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(14-3-3, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Bone morphogenetic proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(2B, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT 5-HT receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(5-HT1, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT 5-HT receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(5-HT3, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(A20, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(ABI-1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(ABI-2, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(ABL-1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ADF (adipocyte differentiation factor), genes for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(APC, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing

promoters)
IT Adenosine receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(A2, A2M2, gene for, expression in wound healing of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Adenosine receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(A3, gene for, expression in wound healing of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Cyclins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(B2, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(BAG-1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(BKLF, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(BRCA1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(BRF1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Bak, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Blk, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Chemokine receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(C-C (cysteine-cysteine chemokine receptors), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(C/EBP (CCAAT box/enhancer element-binding protein), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(CDC25a, expression in wound healing of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(CHOP-10, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)
IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(CRABP-II (cellular retinoic acid binding protein II) gene for,

expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study) (CREB2, gene for, expression in wound healing of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study) (CTCF, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Antigens
RL: BSU (Biological study, unclassified); BIOL (Biological study) (CTLA-1, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process) (CYP1B1, expression in wound healing of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process) (CamK, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Cyclins
RL: BSU (Biological study, unclassified); BIOL (Biological study) (D2, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process) (DAD1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Enzymes, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study) (DNA-repairing, MHR23B, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study) (DP-1, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process) (DSS-1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Prostaglandins
RL: BSU (Biological study, unclassified); BIOL (Biological study) (E, receptors for, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Cadherins
RL: BSU (Biological study, unclassified); BIOL (Biological study) (E-, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study) (EB1 (Epstein-Barr virus 1), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(Elf-1 (gene E74 protein-like factor 1), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Erk1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Cell adhesion molecules
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(F3, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(FLIP (FLICE-like inhibitory protein), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Faf1, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Fli-1, expression in wound healing of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Cyclins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(G, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT GABA receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(GABAA, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(GATA-3, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(GKLF (gut-enriched Kruppel-like factor), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(GRP78 (glucose-regulated protein, 78,000-mol-wt.), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(GTT, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Gadd45, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT GTPase-activating protein
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(GapIII, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal

RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Gli, expression in wound healing of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Phosphoproteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(HMG14, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(HOX8, expression in wound healing of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Heat-shock proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(HSP 27, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Heat-shock proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(HSP 60, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(HSTF (heat-shock transcription factor), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Hck, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Heat-shock proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Hsp84, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Heat-shock proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Hsp88, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Cell adhesion molecules
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ICAM-1 (intercellular adhesion mol. 1), gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Insulin-like growth factor-binding proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(IGF-BP-2, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Insulin-like growth factor-binding proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(IGF-BP-3, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Insulin-like growth factor-binding proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(IGF-BP-4, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Insulin-like growth factor-binding proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(IGF-BP-5, gene for, expression in healing wounds of; identification of loci involved in accelerated wound healing and development of new wound healing promoters)

IT Insulin-like growth factor-binding proteins

=> d bib abs 4

L37 ANSWER 4 OF 8 HCAPLUS COPYRIGHT 2001 ACS
AN 1999:275641 HCAPLUS
DN 131:56979
TI Adhesion of cultured bovine aortic endothelial cells to laminin-1 mediated by **dystroglycan**
AU Shimizu, Hisao; Hosokawa, Hiroshi; Ninomiya, Haruaki; Miner, Jeffrey H.; Masaki, Tomoh
CS Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto, 606, Japan
SO J. Biol. Chem. (1999), 274(17), 11995-12000
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB Expression of **dystroglycan** (DG) by cultured bovine aortic endothelial (BAE) cells was confirmed by cDNA cloning from a BAE cDNA library, Northern blotting of mRNA, Western blotting of membrane proteins, and double immunostaining with **antibodies** against .beta.DG and platelet endothelial cell adhesion mol.-1. Immunocytochem. **anal**. revealed localization of DG in multiple plaques on the basal side of resting cells. This patchy distribution was obscured in migrating cells, in which the most prominent staining was obsd. in the trailing edge anchoring the cells to the substratum. Biotin-labeled laminin-1 overlay **assay** of dissocd. BAE membrane proteins indicated the interaction of laminin-1 with .alpha.DG. The laminin .alpha.5 globular domain fragment expressed in bacteria and labeled with biotin could also bind .alpha.DG on the membrane blot, and the unlabeled fragment disrupted the binding of biotin-laminin-1 to .alpha.DG. The interaction of biotin-laminin-1 with .alpha.DG was inhibited by sol. .alpha.DG contained in the conditioned medium from DG cDNA-transfected BAE cells and by a series of glycosaminoglycans (heparin, dextran sulfate, and fucoidan). Sol. .alpha.DG in the conditioned medium inhibited the adhesion of BAE cells to laminin-1-coated dishes, whereas it had no effect on their adhesion to fibronectin. All three glycosaminoglycans that disrupted the biotin-laminin-1 binding to .alpha.DG inhibited BAE cell adhesion to laminin-1, whereas they failed to inhibit the adhesion to fibronectin. These results indicate a role of DG as a non-integrin laminin receptor involved in vascular endothelial cell adhesion to the extracellular matrix.
RE.CNT 46
RE
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(2) Azizkhan, R; J Exp Med 1980, V152, P931 HCAPLUS
(3) Basson, C; J Cell Biol 1990, V110, P789 HCAPLUS
(4) Belkin, A; Cell Adhes Commun 1996, V4, P281 HCAPLUS
(5) Bowe, M; Neuron 1994, V12, P1173 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 5

L37 ANSWER 5 OF 8 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:600577 HCAPLUS
DN 129:312375
TI Localization of dystrophin isoform Dp71 to the inner limiting membrane of the retina suggests a unique functional contribution of Dp71 in the retina
AU Howard, Perry L.; Dally, Ghassan Y.; Wong, Melanie H.; Ho, Alex; Weleber, Richard G.; Pillers, De-Ann M.; Ray, Peter N.
CS Dep. Med. Mol. Genetics, Univ. Toronto, Toronto, ON, Can.
SO Hum. Mol. Genet. (1998), 7(9), 1385-1391
CODEN: HMGEE5; ISSN: 0964-6906
PB Oxford University Press
DT Journal
LA English
AB The electroretinograms (ERGs) of patients with Duchenne muscular dystrophy and an allelic variant of the mdx mouse (mdxCv3) have been shown to be abnormal. **Anal.** of five allelic variants of the mdx mouse with mutations in the dystrophin gene has shown that there is a correlation between the position of the mutation and the severity of the ERG abnormality. Three isoforms are expressed in the retina: Dp427, Dp260 and Dp71. Using indirect immunofluorescence and isoform-specific **antibodies** on retinal sections from three allelic mdx mouse strains, the authors have examd. the localization of each of the isoforms. The authors show that Dp71 expression does not overlap with Dp427 and Dp260 expression at the outer plexiform layer (OPL). Instead, Dp71 is localized to the inner limiting membrane (ILM) and to retinal **blood** vessels. Moreover, the authors show that Dp260 and Dp71 differ structurally at their resp. C-termini. In addn., the authors find that the proper localization of the .beta.-**dystroglycan** is dependent upon both Dp260 and Dp71 are non-redundant isoforms that are located at different sites within the retina yet have a common interaction with .beta.-**dystroglycan**. These data suggest that both Dp71 and Dp260 contribute distinct but essential roles to retinal electrophysiol.

=> d bib abs 6

L37 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2001 ACS
AN 1996:572967 HCAPLUS
DN 125:271478
TI Expression of a dystrophin-sarcoglycan complex in **serum**-deprived BC3H1 cells and involvement of .alpha.-sacroglycan in substrate attachment
AU Yoshida, Tomokazu; Hanada, Hironori; Iwata, Yuko; Pan, Yan; Sigekawa, Munekazu
CS Dep. Mol. Physiology, National Cardiovascular Center Res. Inst., Osaka, 565, Japan
SO Biochem. Biophys. Res. Commun. (1996), 225(1), 11-15
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English
AB Dystrophin and .alpha.- and .gamma.-sarcoglycans were newly expressed in BC3H1 cells during differentiation induced by **serum** withdrawal. These proteins formed a tight complex with other dystrophin-assocd. proteins (DAPs), as **detected** by immunopptn. with anti-dystrophin **antibody**. Integrins .beta.1 and .beta.3, vinculin, and focal adhesion kinase were also **detected** in the same immunoppt. In a cell adhesion **assay**, differentiated BC3H1 cells attached more efficiently to type I collagen-coated dishes than nondifferentiated cells and loss of .alpha.-sarcoglycan induced by antisense oligodeoxynucleotide in differentiated cells resulted in significant inhibition of cell adhesion. Thus dystrophin and DPAs, at least partly, form a complex with the focal adhesion proteins in differentiated BC3H1 cells and .alpha.-sarcoglycan seems to modulate the function of the focal adhesion complex in these cells.

=> d bib abs 7

L37 ANSWER 7 OF 8 HCAPLUS COPYRIGHT 2001 ACS
AN 1996:381589 HCAPLUS
DN 125:82610
TI Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins
AU Song, Kenneth S.; Scherer, Philipp E.; Tang, ZhaoLan; Okamoto, Takashi; Li, Shengwen; Chafel, Mark; Chu, Caryn; Kohtz, Stave; Lisanti, Michael P.
CS Whitehead Institute Biomedical Research, Cambridge, MA, 02142-1479, USA
SO J. Biol. Chem. (1996), 271(25), 15160-15165
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Caveolae are microdomains of the plasma membrane that have been implicated in signal transduction. Caveolin, a 21-24-kDa integral membrane protein, is a principal component of the caveolae membrane. Recently, we and others have identified a family of caveolin-related proteins; caveolin has been retermed caveolin-1. Caveolin-3 is most closely related to caveolin-1, but caveolin-3 mRNA is expressed only in muscle tissue types. Here, we examine (i) the expression of caveolin-3 protein in muscle tissue types and (ii) its localization within skeletal muscle fibers by immunofluorescence microscopy and subcellular fractionation. For this purpose, we generated a novel monoclonal **antibody** (mAb) probe that recognizes the unique N-terminal region of caveolin-3, but not other members of the caveolin gene family. A survey of tissues and muscle cell types by Western blot **anal.** reveals that the caveolin-3 protein is selectively expressed only in heart and skeletal muscle tissues, cardiac myocytes, and smooth muscle cells. Immunolocalization of caveolin-3 in skeletal muscle fibers demonstrates that caveolin-3 is localized to the sarcolemma (muscle cell plasma membrane) and coincides with the distribution of another muscle-specific plasma membrane marker protein, dystrophin. In addn., caveolin-3 protein expression is dramatically induced during the differentiation of C2C12 skeletal myoblasts in culture. Using differentiated C2C12 skeletal myoblasts as a model system, we observe that caveolin-3 co-fractionates with cytoplasmic signaling mols. (G-proteins and Src-like kinases) and members of the dystrophin complex (dystrophin, .alpha.-sarcoglycan, and .beta.-**dystroglycan**), but is clearly sepd. from the bulk of cellular proteins. Caveolin-3 co-immunoppts. with **antibodies** directed against dystrophin, suggesting that they are phys. assocd. as a discrete complex. These results are consistent with previous immunoelectron microscopic studies demonstrating that dystrophin is localized to plasma membrane caveolae in smooth muscle cells.

=> d bib abs 8

L37 ANSWER 8 OF 8 HCAPLUS COPYRIGHT 2001 ACS
AN 1995:651287 HCAPLUS
DN 123:52954
TI Non-muscle .alpha.-**dystroglycan** is involved in **epithelial** development
AU Durbeej, Madeleine; Larsson, Erik; Ibraghimov-Beskrovnaya, Oxana; Roberds, Steven L.; Campbell, Kevin P.; Ekblom, Peter
CS Dep. Animal Physiology, Uppsala Univ., Uppsala, Swed.
SO J. Cell Biol. (1995), 130(1), 79-91
CODEN: JCLBA3; ISSN: 0021-9525
DT Journal
LA English
AB The **dystroglycan** complex is a transmembrane linkage between the cytoskeleton and the basement membrane in muscle. One of the components of the complex, .alpha.-**dystroglycan** binds both laminin of muscle (laminin-2) and agrin of muscle basement membranes. **Dystroglycan** has been **detected** in nonmuscle tissues as well, but the physiol. role in nonmuscle tissues has remained unknown. Here the authors show that **dystroglycan** during mouse development in nonmuscle tissues is expressed in epithelium. In situ by hybridization revealed strong expression of **dystroglycan** mRNA in all studied **epithelial** sheets, but not in endothelium or mesenchyme. Conversion of mesenchyme to epithelium occurs during kidney development, and the embryonic kidney was used to study the role of .alpha.-**dystroglycan** for **epithelial** differentiation. During in vitro culture of the metanephric mesenchyme, the first morphol. signs of **epithelial** differentiation can be seen on day two. Northern blots revealed a clear increase in **dystroglycan** mRNA on day two of in vitro development. A similar increase of expression on day two was previously shown for laminin .alpha.1 chain. Immunofluorescence showed that **dystroglycan** is strictly located on the basal side of developing kidney **epithelial** cells. Monoclonal **antibodies** known to block binding of .alpha.-**dystroglycan** to laminin-1 perturbed development of epithelium in kidney organ culture, whereas control **antibodies** did not do so. The authors suggest that the **dystroglycan** complex acts as a receptor for basement membrane components during **epithelial** morphogenesis. It is likely that this involves binding of .alpha.-**dystroglycan** to E3 fragment of laminin-1.

=> d bib abs l46 1-9

L46 ANSWER 1 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 2001:236513 HCAPLUS
DN 134:338699
TI Regulation of laminin 1-induced pancreatic .beta.-cell differentiation by .alpha.6 integrin and .alpha.-**dystroglycan**
AU Jiang, Fang-Xu; Georges-Labouesse, E.; Harrison, Leonard C.
CS Autoimmunity and Transplantation Division, The Walter and Eliza Hall Institute of Medical Research, The Royal Melbourne Hospital, Parkville, 3050, Australia
SO Mol. Med. (Baltimore, MD, U. S.) (2001), 7(2), 107-114
CODEN: MOMEF3; ISSN: 1076-1551
PB Johns Hopkins University Press
DT Journal
LA English
AB The ability to manipulate the development of pancreatic insulin-producing .beta. cells has implications for the treatment of type 1 diabetes. Previously, we found that laminin-1, a basement membrane trimeric glycoprotein, promotes .beta.-cell differentiation. We have investigated the mechanism of this effect, using agents that block the receptors for laminin-1, .alpha.6 integrin, and .alpha.-**dystroglycan** (.alpha.-**DG**). Dissocd. cells from 13.5-day postcoitum (dpc) fetal mouse pancreas were cultured for 4 days with laminin-1, with and without monoclonal **antibodies** and other agents known to block integrins or .alpha.-**DG**. Fetuses fixed in Bouin's soln. or fetal pancreas cells fixed in 4% paraformaldehyde were processed for routine histol. and for immunohistol. to **detect** hormone expression and bromodeoxyuridine (BrdU) uptake. Blocking the binding of laminin-1 to .alpha.6 integrin with a monoclonal **antibody**, GoH3, abolished cell proliferation (BrdU uptake) and doubled the no. of .beta. cells. Inhibition of mols. involved in .alpha.6 integrin signaling (phosphatidylinositol 3-kinase, F-actin, or mitogen-activated protein kinase) had a similar effect. Nevertheless, .beta. cells appeared to develop normally in .alpha.6 integrin-deficient fetuses. Blocking the binding of laminin-1 to .alpha.-**DG** with a monoclonal **antibody**, IIH6, dramatically decreased the no. of .beta. cells. Heparin, also known to inhibit laminin-1 binding to .alpha.-**DG**, had a similar effect. In the presence of heparin, the increase in .beta. cells in response to blocking .alpha.6 integrin with GoH3 was abolished. These findings reveal an interplay between .alpha.6 integrin and .alpha.-**DG** to regulate laminin-1-induced .beta.-cell development. Laminin-1 had a dominant effect via .alpha.-**DG** to promote cell survival and .beta.-cell differentiation, which was modestly inhibited by .alpha.6 signaling.
RE.CNT 42
RE
(2) Alpert, S; Cell 1988, V53, P295 HCAPLUS
(3) Bosco, D; Diabetes 2000, V49, P233 HCAPLUS
(4) Brown, S; J Cell Sci 1999, V112, P209 HCAPLUS
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(8) De Arcangelis, A; Development 1999, V126, P3957 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 2001:147740 HCAPLUS
DN 134:264323
TI Airway **epithelial** cell wound repair mediated by .alpha.-**dystroglycan**
AU White, Steven R.; Wojcik, Kimberly R.; Gruenert, Dieter; Sun, Steven; Dorscheid, Delbert R.
CS Section of Pulmonary and Critical Care Medicine, Department of Medicine, Division of Biological Sciences, University of Chicago, Chicago, IL, 60637, USA
SO Am. J. Respir. Cell Mol. Biol. (2001), 24(2), 179-186
CODEN: AJRBEL; ISSN: 1044-1549
PB American Thoracic Society
DT Journal
LA English
AB **Dystroglycans** (**DGs**) bind laminin matrix proteins in

skeletal and cardiac muscle and are expressed in other nonmuscle tissues. However, their expression in airway **epithelial** cells has not been demonstrated. We examd. expression of **DGs** in the human airway **epithelial** cell line 1HAEo-, and in human primary airway **epithelial** cells. Expression of the common gene for .alpha.- and .beta.-**DG** was demonstrated by reverse transcriptase/polymerase chain reaction in 1HAEo- cells. Protein expression of .beta.-**DG** was demonstrated by both Western blot and flow cytometry in cultured cells. Localization of .alpha.-**DG**, using both a monoclonal **antibody** and the .alpha.-**DG** binding lectin wheat-germ agglutinin (WGA), was to the cell membrane and nucleus. We then examd. the function of **DGs** in modulating wound repair over laminin matrix. Blocking .alpha.-**DG** binding to laminin in 1HAEo- monolayers using either **glycosaminoglycans** or WGA attenuated cell migration and spreading after mech. injury. .alpha.-**DG** was not expressed in **epithelial** cells at the wound edge immediately after wound creation, but localized to the cell membrane in these cells within 12 h of injury. These data demonstrate the presence of **DGs** in airway epithelium. .alpha.-**DG** is dynamically expressed and serves as a lectin to bind laminin during airway **epithelial** cell repair.

RE.CNT 43

RE

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(2) Andac, Z; J Mol Biol 1999, V287, P253 HCAPLUS
(3) Belkin, A; Cell Adhes Commun 1996, V4, P281 HCAPLUS
(4) Campanelli, J; Cell 1994, V77, P663 HCAPLUS
(5) Campbell, K; Cell 1995, V80, P675 HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:288094 HCAPLUS
DN 133:56446
TI .alpha.-**dystroglycan** isoforms are differentially distributed in adult rat retina
AU Moukhles, Hakima; Roque, Rouel; Carbonetto, Salvatore
CS Centre for Research in Neuroscience, McGill University and Montreal General Hospital Research Institute, Montreal, PQ, H3G 1A4, Can.
SO J. Comp. Neurol. (2000), 420(2), 182-194
CODEN: JCNEAM; ISSN: 0021-9967
PB Wiley-Liss, Inc.
DT Journal
LA English
AB .alpha.-**Dystroglycan** (.alpha.-**DG**) is a laminin/agrin receptor expressed in skeletal muscle as well as in nervous system and other tissues. Glycosylation of the core protein of .alpha.-**DG** is extensive, variable from tissue to tissue, and functionally relevant. To address differential glycosylation of .alpha.-**DG** in the retina, we have investigated the distribution of this protein using two different **antibodies**: 1B7 directed against the core protein of .alpha.-**dystroglycan**, and IIH6 directed against a carbohydrate moiety. Monoclonal **antibody** 1B7 recognizes a broader band than IIH6, which seems to recognize only a subset of .alpha.-**DG** forms in retina. These data reflect the existence of differentially glycosylated isoforms of .alpha.-**DG**. Monoclonal **antibody** 1B7 shows an extensive staining for .alpha.-**DG** in the inner limiting membrane as well as in the ganglion cell and inner plexiform layers labeling Muller cell processes, whereas monoclonal **antibody** IIH6 staining is restricted to the inner limiting membrane and **blood** vessels. Our data indicate that there are distinct isoforms of .alpha.-**DG** that are localized in apposition to basal lamina in the inner limiting membrane and **blood** vessels or within the parenchyma of the retina along Muller glia. Both isoforms are expressed in a Muller cell line in culture and coimmunoppt. with .beta.-**dystroglycan**. These data suggest that **DGs** may participate in organizing synapses and basement membrane assembly in the retina.

RE.CNT 58

RE

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(2) Belkin, A; Cell Adhes Commun 1996, V4, P281 HCAPLUS
(3) Blake, D; Proc Natl Acad Sci USA 1998, V95, P241 HCAPLUS
(4) Blank, M; J Comp Neurol 1997, V389, P668 HCAPLUS
(5) Bowe, M; Neuron 1994, V12, P1173 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 2000:5275 HCAPLUS
DN 132:135067
TI Neural regulation of .alpha.-**dystroglycan** biosynthesis and glycosylation in skeletal muscle
AU Leschziner, Andres; Moukhles, Hakima; Lindenbaum, Michael; Gee, Stephen H.; Butterworth, Joanne; Campbell, Kevin P.; Carbonetto, Salvatore
CS Centre for Research in Neuroscience, Montreal General Hospital Research Institute, McGill University, Montreal, PQ, Can.
SO J. Neurochem. (2000), 74(1), 70-80
CODEN: JONRA9; ISSN: 0022-3042
PB Lippincott Williams & Wilkins
DT Journal
LA English
AB .alpha.-**Dystroglycan** (.alpha.-**DG**) is part of a complex of cell surface proteins linked to dystrophin or utrophin, which is distributed over the myofiber surface and is concd. at neuromuscular junctions. In laminin overlays of muscle exts. from developing chick hindlimb muscle, .alpha.-**DG** first appears at embryonic day (E) 10 with an apparent mol. mass of 120 kDa. By E 15 it is replaced by smaller (.apprx.100 kDa) and larger (.apprx.150 kDa) isoforms. The larger form increases in amt. and in mol. mass (>200 kDa) as the muscle is innervated and the postsynaptic membrane differentiates (E10-E20), and then decreases dramatically in amt. after hatching. In myoblasts differentiating in culture the mol. mass of .alpha.-**DG** is not significantly increased by their replication, fusion, or differentiation into myotubes. Monoclonal **antibody** IIH6, which recognizes a carbohydrate epitope on .alpha.-**DG**, preferentially binds to the larger forms, suggesting that the core protein is differentially glycosylated beginning at E16. Consistent with prior observations implicating the IIH6 epitope in laminin binding, the smaller forms of .alpha.-**DG** bind more weakly to laminin affinity columns than the larger ones. In blots of adult rat skeletal muscle probed with radiolabeled laminin or monoclonal **antibody** IIH6, .alpha.-**DG** appears as a >200-kDa band that decreases in mol. mass but increases in intensity following denervation. Northern blots reveal a single mRNA transcript, indicating that the redn. in mol. mass of .alpha.-**DG** after denervation is not obviously a result of alternative splicing but is likely due to posttranslational modification of newly synthesized mols. The regulation of .alpha.-**DG** by the nerve and its increased affinity for laminin suggest that glycosylation of this protein may be important in myofiber-basement membrane interactions during development and after denervation.
RE.CNT 77
RE
(1) Bewick, G; Neuroreport 1992, V3, P857 HCAPLUS
(2) Biral, D; J Muscle Res Cell Moril 1996, V17, P523 HCAPLUS
(4) Bowe, M; Neuron 1994, V12, P1173 HCAPLUS
(7) Burden, S; Dev Biol 1977, V57, P317 HCAPLUS
(8) Campanelli, J; Cell 1994, V77, P663 HCAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L46 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:542280 HCAPLUS
DN 129:242955
TI .alpha.-**dystroglycan** functions in acetylcholine receptor aggregation but is not a coreceptor for agrin-MuSK signaling
AU Jacobson, Christian; Montanaro, Federica; Lindenbaum, Michael; Carbonetto, Salvatore; Ferns, Michael
CS Departments of Biology and Neurology and Neurosurgery, McGill University and the Centre for Research in Neuroscience, Montreal, PQ, H3G 1A4, Can.
SO J. Neurosci. (1998), 18(16), 6340-6348
CODEN: JNRSDS; ISSN: 0270-6474
PB Society for Neuroscience

DT Journal
LA English
AB .alpha.-**Dystroglycan** (.alpha.-**DG**) is an agrin-binding protein that has been implicated in acetylcholine receptor (AChR) clustering, but it is unclear whether it acts as a coreceptor involved in initial agrin signaling or as a component involved in later events. To investigate its role, we have generated antisense derivs. of the C2 mouse muscle cell line, which have reduced .alpha.-**DG** expression. When compared with wild-type cells, the .alpha.-**DG**-deficient myotubes have a dramatic redn. in the no. of spontaneous and agrin-induced AChR clusters. Several findings suggest that this decrease in AChR clustering is likely not because of a defect in agrin signaling through the MuSK receptor tyrosine kinase. Compared with wild-type cells, the .alpha.-**DG**-deficient cell lines showed only a transient redn. in the level of agrin-induced MuSK tyrosine phosphorylation and no redn. in AChR .beta.-subunit tyrosine phosphorylation. Addnl., agrin-induced phosphorylation of MuSK in wild-type myotubes was not decreased using agrin fragments that lack the domain primarily responsible for binding to .alpha.-**DG**. Finally, neural agrin-induced phosphorylation of MuSK was unaffected by treatments such as excess muscle agrin or anti-.alpha.-**DG antibodies**, both of which block agrin-.alpha.-**DG** binding. Together, these results suggest that .alpha.-**DG** is not required for agrin-MuSK signaling but rather that it may play a role elsewhere in the clustering pathway, such as in the downstream consolidation or maintenance of AChR clusters.

L46 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 1998:93784 HCAPLUS
DN 128:215920
TI Laminin and .alpha.-**dystroglycan** mediate acetylcholine receptor aggregation via a MuSK-independent pathway
AU Montanaro, Federica; Gee, Stephen H.; Jacobson, Christian; Lindenbaum, Michael H.; Froehner, Stanley C.; Carbonetto, Salvatore
CS Centre for Research in Neuroscience, Montreal General Hospital Research Institute, McGill University, Montreal, PQ, H3G 1A4, Can.
SO J. Neurosci. (1998), 18(4), 1250-1260
CODEN: JNRSDS; ISSN: 0270-6474
PB Society for Neuroscience
DT Journal
LA English
AB Specific isoforms of laminin (LN) are concd. at neuromuscular junctions (NMJs) where they may participate in synaptic organization or function. In myotubes from C2 cells, LN is concd. within the majority of spontaneous acetylcholine receptor (AChR) aggregates. Neural agrin substantially increases this colocalization, suggesting that agrin can recruit LN into AChR aggregates. Addn. of LN to C2 myotubes induces a more than twofold increase in the no. of AChR aggregates. These aggregates have a larger size and are more dense than are those induced by agrin, suggesting that LN is involved in the growth and/or stabilization of AChR aggregates. Consistent with this hypothesis, an antiserum to LN reduces the size of individual AChR aggregates but increases their no. In C2 myotubes, extracellular matrix receptors contg. the integrin .beta.1 subunit are poorly colocalized with AChR aggregates, suggesting that integrins may not be involved in LN-induced aggregation. In contrast, almost all AChR aggregates are assocd. with **dystroglycan** immunoreactivity, and monoclonal **antibody** (mAb) IIH6 against .alpha.-**dystroglycan** (.alpha.-**DG**), a LN and agrin receptor, causes a concn.-dependent inhibition of LN-induced aggregation. Moreover, S27 cells, which lack a functional .alpha.-**DG**, and two C2-derived cell lines expressing antisense **DG** mRNA fail to aggregate AChRs in response to LN. Finally, LN-induced AChR aggregation does not involve the phosphorylation of the muscle-specific tyrosine kinase receptor (MuSK) or the AChR .beta. subunit. We hypothesize that the interaction of LN with .alpha.-**DG** contributes to the growth and/or stabilization of AChR microaggregates into macroaggregates at the developing NMJ via a MuSK-independent mechanism.

L46 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 1997:182758 HCAPLUS
DN 126:261931

TI Laminin-induced clustering of **dystroglycan** on embryonic muscle cells: comparison with agrin-induced clustering
AU Cohen, Monroe W.; Jacobson, Christian; Yurchenco, Peter D.; Morris, Glenn E.; Carbonetto, Salvatore
CS Department of Physiology, McGill University, Montreal, PQ, H3G1Y6, Can.
SO J. Cell Biol. (1997), 136(5), 1047-1058
CODEN: JCLBA3; ISSN: 0021-9525
PB Rockefeller University Press
DT Journal
LA English
AB The effect of laminin on the distribution of **dystroglycan** (**DG**) and other surface proteins was examd. by fluorescent staining in cultures of muscle cells derived from Xenopus embryos. Western blotting confirmed that previously characterized **antibodies** are reactive in Xenopus. In control cultures, .alpha.**DG**, .beta. **DG**, and laminin binding sites were distributed as microclusters (<1 .mu.m2 in area) over the entire dorsal surface of the muscle cells. Treatment with laminin induced the formation of macroclusters (1-20 .mu.m2), accompanied by a corresponding decline in the d. of the microclusters. With 6 nM laminin, clustering was apparent within 150 min and near maximal within 1 day. Laminin was effective at 30 pM, the lowest concn. tested. The laminin fragment E3, which competes with laminin for binding to .alpha.**DG**, inhibited laminin-induced clustering but did not itself cluster **DG**, thereby indicating that other portions of the laminin mol. in addn. to its .alpha.**DG** binding domain are required for its clustering activity. Laminin-induced clusters also contained dystrophin, but unlike agrin-induced clusters, they did not contain acetylcholine receptors, utrophin, or phosphotyrosine, and their formation was not inhibited by a tyrosine kinase inhibitor. The results reinforce the notion that unclustered **DG** is mobile on the surface of embryonic muscle cells and suggest that this mobile **DG** can be trapped by .gtoreq.2 different sets of mol. interactions. Laminin self binding may be the basis for the laminin-induced clustering.

L46 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 1996:757053 HCAPLUS
DN 126:29935
TI The **sarcoglycan** complex in the six autosomal recessive limb-girdle muscular dystrophies
AU Vainzof, M.; Passos-Bueno, M. R.; Canovas, M.; Moreira, E. S.; Pavanello, R. C. M.; Marie, S. K.; Anderson, L. V. B.; Bonnemann, C. G.; McNally, E. M.; Nigro, V.; Kunkel, L. M.; Zatz, M.
CS Dep. Biol., IB-USP, Sao Paulo, 05508-900, Brazil
SO Hum. Mol. Genet. (1996), 5(12), 1963-1969
CODEN: HMGEE5; ISSN: 0964-6906
PB Oxford University Press
DT Journal
LA English
AB To enhance our understanding of the autosomal recessive limb-girdle muscular dystrophy (LGMD), patients from six genetically distinct forms (LGMD2A to LGMD2F) were studied with **antibodies** directed against four **sarcoglycan** subunits (.alpha.-, .beta.-, .gamma.-, .delta.-SG), dystrophin, .beta.-**dystroglycan** (.beta.-**DG**) and merosin. All patients with LGMD2A and 2B had a mild clin. course while those with a primary **sarcoglycan** mutation (LGMD2C to 2F) had a range of clin. severity. The dystrophin and merosin immunofluorescence patterns were pos. in patients with all six AR LGMDs. The majority of patients with a severe Duchenne-like phenotype presented total absence of the SG complex. However, some exceptions were found in 13q linked patients, indicating that the presence of a certain labeling for components of the SG may not be prognostic for a milder phenotype. The observation that the primary absence of .alpha.-SG results in the total absence of .beta.- and .delta.-SG but not of .gamma.-SG suggests that the .alpha.-, .beta.- and .delta.-subunits of **sarcoglycan** may be more closely assocd. A secondary redn. in dystrophin amt. was seen in patients with primary **sarcoglycan** mutations, which was most marked in patients with primary .beta.-, .gamma.-, and .delta.-SG deficiencies. In contrast, .beta.-**DG** staining was retained in all patients, suggesting that the assocn. between SG and **DG** subcomplexes is not so strong. Based on the above findings, the authors

have refined the model for the interaction among the known glycoproteins of the **sarcoglycan** complex, within the DGC.

L46 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2001 ACS
AN 1996:454736 HCAPLUS
DN 125:138282
TI Non-neural agrin codistributes with acetylcholine receptors during early differentiation of Torpedo electrocytes
AU Cartaud, A.; Ludosky, M. A.; Haasemann, M.; Jung, D.; Campbell, K.; Cartaud, J.
CS Departement de Biologie Supramoleculaire et Cellulaire, CNRS, Paris, 75251, Fr.
SO J. Cell Sci. (1996), 109(7), 1837-1846
CODEN: JNCSAI; ISSN: 0021-9533
DT Journal
LA English
AB Agrin, an extracellular matrix protein synthesized in nerves and muscles is known to promote the clustering of acetylcholine receptors and other synaptic proteins in cultured myotubes. This observation suggests that agrin may provide at least part of the signal for synaptic specialization in vivo. The extracellular matrix components agrin, laminin and merosin bind to .alpha.-**dystroglycan**, a heavily glycosylated peripheral protein part of the dystrophin-glycoprotein complex, previously characterized in the sarcolemma of skeletal and cardiac muscle and at the neuromuscular junction. In order to understand further the function of agrin and .alpha.**DG** in the genesis of the acetylcholine receptor-rich membrane domain, the settling of components of the dystrophin-glycoprotein complex and agrin was followed by immunofluorescence localization in developing Torpedo marmorata electrocytes. In 40-45 mm Torpedo embryos, a stage of development at which the electrocytes exhibit a definite structural polarity, dystrophin, .alpha./.beta.-**dystroglycan** and agrin accumulated concomitantly with acetylcholine receptors at the ventral pole of the cells. Among these components, agrin appeared as the most intensely concd. and sharply localized. The scarcity of the nerve-electrocyte synaptic contacts at this stage of development, monitored by **antibodies** against synaptic vesicles, further indicates that before innervation, the machinery for acetylcholine receptor clustering is provided by electrocyte-derived agrin rather than by neural agrin. These observations suggest a two-step process of acetylcholine receptor clustering involving: an instructive role of electrocyte-derived agrin in the formation of a dystrophin-based membrane scaffold upon which acetylcholine receptor mols. would accumulate according to a diffusion trap model; and a maturation and/or stabilization step controlled by neural agrin. In light of these data, the existence of more than one agrin receptor is postulated to account for the action of the agrin variants at different stages of the differentiation of the postsynaptic membrane in Torpedo electrocytes.

=> d bib abs 1

L53 ANSWER 1 OF 14 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2001-091717 [10] WPIDS
DNN N2001-069461 DNC C2001-027106
TI **Diagnosing** the **tumorigenic** grade of a malignant tissue for e.g. grading human prostatic and breast **adenocarcinoma**, comprises **measuring** the amount of **dystroglycan** protein in a **tumor** tissue.
DC B04 D16 S03
IN CAMPBELL, K P; COHEN, M B; HENRY, M
PA (IOWA) UNIV IOWA RES FOUND
CYC 90
PI WO 2001001151 A2 20010104 (200110)* EN 18p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2000064040 A 20010131 (200124)
ADT WO 2001001151 A2 WO 2000-US40206 20000615; AU 2000064040 A AU 2000-64040 20000615
FDT AU 2000064040 A Based on WO 200101151
PRAI US 1999-141149 19990625
AN 2001-091717 [10] WPIDS
AB WO 200101151 A UPAB: 20010220
NOVELTY - **Diagnosing** the **tumorigenic** grade of a malignant tissue comprises **determining** the amount of **dystroglycan** protein (I) in the tissue relative to a standard.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a prognostic method for malignancy comprising **measuring** the expression level of the **dystroglycan** gene (II) in a sample of malignant tissue and comparing the result with a control, any decrease indicating a poor prognosis;

(2) identifying subjects at risk of development (or recurrence after treatment) of **cancer** where a decreased level of (II) expression relative to a control indicates a subject at high risk;

(3) identifying subjects at risk of developing **cancer** by screening for mutations in (II); and

(4) **detecting cancer** in a tissue by **detecting** a decreased level of (I) in a sample relative to a standard.

ACTIVITY - Cytostatic. No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - The method is particularly used to grade human prostatic and breast **adenocarcinoma**, also for prognosis of **tumors**, identification of subjects at risk of development (or recurrence) of **tumors** and for **detecting cancer**. Subjects at risk may also be identified by **detecting** mutations in the **dystroglycan** gene. Expression of (I) is reduced, or undetectable, in **tumor** cells with the extent of the reduction being greatest in high-grade, invasive **cancers**. Also, the ability of **cancer** cells to metastatize can be inhibited by introducing a functional **dystroglycan** gene, optionally under control of a **tumor**-specific promoter, e.g. in an adenoviral vector.

Dwg.0/0

=> d bib abs 2

L53 ANSWER 2 OF 14 MEDLINE
AN 2001182434 MEDLINE
DN 21094555 PubMed ID: 11159052
TI Airway epithelial cell wound repair mediated by alpha-**dystroglycan**
.
AU White S R; Wojcik K R; Gruenert D; Sun S; Dorscheid D R
CS Section of Pulmonary and Critical Care Medicine, Department of Medicine, University of Chicago, 5841 S. Maryland Ave., MC6076, Chicago, IL 60637, USA.. swhite@medicine.bsd.uchicago.edu
NC HL-07605 (NHLBI)
HL-51853 (NHLBI)
HL-60531 (NHLBI)
SO AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY, (2001 Feb) 24 (2) 179-86.
Journal code: AOB; 8917225. ISSN: 1044-1549.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200103
ED Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010329
AB **Dystroglycans** (DGs) bind **laminin** matrix proteins in skeletal and cardiac muscle and are expressed in other nonmuscle tissues. However, their expression in airway epithelial cells has not been demonstrated. We examined expression of DGs in the human airway epithelial cell line 1HAEo(-), and in human primary airway epithelial cells. Expression of the common gene for alpha- and beta-DG was demonstrated by reverse transcriptase/ polymerase chain reaction in 1HAEo(-) cells. Protein expression of beta-DG was demonstrated by both Western blot and flow cytometry in cultured cells. Localization of alpha-DG, using both a **monoclonal antibody** and the alpha-DG binding lectin wheat-germ agglutinin (WGA), was to the cell membrane and nucleus. We then examined the function of DGs in modulating wound repair over **laminin** matrix. Blocking alpha-DG binding to **laminin** in 1HAEo(-) monolayers using either glycosyaminoglycans or WGA attenuated cell migration and spreading after mechanical injury. alpha-DG was not expressed in epithelial cells at the wound edge immediately after wound creation, but localized to the cell membrane in these cells within 12 h of injury. These data demonstrate the presence of DGs in airway epithelium. alpha-DG is dynamically expressed and serves as a lectin to bind **laminin** during airway epithelial cell repair.

=> d bib abs 3

L53 ANSWER 3 OF 14 USPATFULL
AN 2000:170867 USPATFULL
TI Neuregulin response element and uses therefor
IN Goldman, Daniel, Ann Arbor, MI, United States
Sapru, Mohan K., Naperville, IL, United States
PA The Regents of the University of Michigan, Ann Arbor, MI, United States (U.S. corporation)
PI US 6162641 20001219
AI US 1998-92636 19980605 (9)
PRAI US 1997-48847 19970606 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Guzo, David; Assistant Examiner: Shuman, Jon
LREP Lahive & Cockfield LLP
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 2496
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for therapeutics and for screens are provided using a 15 bp sequence in the rat .epsilon.-subunit promoter that regulates PTPase, neuregulin and Ras-dependent gene expression. As this 15 bp sequence is necessary also for low .epsilon.-subunit gene expression in extrajunctional regions of the muscle fiber, the screens can identify agents that simultaneously and oppositely modulate expression in .epsilon.-subunit expression of synaptic and extrajunctional regions.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic 3

L53 ANSWER 3 OF 14 USPATFULL

SUMM . . . have functional activity at a synapse. Genes included among those that are desirable to express at a synapse include agrin, **laminin** .beta.-2, **dystroglycan**, rapsyn, utrophin or MuSK. The method of contacting a host cell with the construct can be used to introduce the. . .

SUMM . . . NGF, NT-3 or NT-4/5. Alternatively, the gene may encode a protein having functional activity at a synapse, for example, agrin, **laminin** .beta.2, **dystroglycan**, rapsyn, utrophin and MuSK.

SUMM . . . recombinant expression vector; introducing the recombinant expression vector into a host cell; exposing the host cell to a candidate compound; **measuring** the reporter gene activity in the presence of said candidate compound; and comparing the reporter gene activity in the presence of said compound with the activity in the absence of said compound, to **determine** whether said candidate compound is an agent capable of regulating synaptic-specific transcription of a gene operably linked to an NRE.

SUMM . . . with an amount of a neuregulin sufficient to induce neuregulin-dependent gene transcription; exposing the host cell to a candidate compound; **measuring** the reporter gene activity in the presence of said candidate compound; and comparing the reporter gene activity in the presence. . .

SUMM . . . together or separately; introducing the recombinant expression vector into a host cell; contacting the host cell with a candidate compound; **measuring** the reporter gene activity in the presence of said compound; and comparing the reporter gene activity in the presence of. . .

DRWD . . . of transfection), in the presence and absence of PTP CL100. Cells were harvested 24 h post-transfection for luciferase and CAT **assays**. The middle panel shows data from samples of L6 stable rat muscle cells, each co-transfected with the indicated .epsilon.-promoter/luciferase expression. . . induction of differentiation, cells were treated with buffer or recombinant neuregulin (5 nM) for 60 h prior to harvesting, and **assayed** for luciferase and chloramphenicol acetyltransferase (CAT) activities. The right panel shows primary myotube cultures co-transfected with the indicated .epsilon.-promoter/luciferase expression. . . medium (Dulbeco's Modified Eagle Medium, DMEM, with 0.5% fetal calf serum, FCS) for 48 h, and were to harvested and **assayed** for luciferase and CAT expression. Experiments were repeated at least three times. Bar graphs represent the average of triplicate transfections. . .

DETD . . . is operatively linked to a gene encoding a protein that enhances formation of a neuromuscular synapse, for example, rapsyn, utrophin, **laminin** .beta.-2, **dystroglycan**, MuSK, and agrin and similar components of synapses in nerve and muscle tissue.

DETD . . . has had loss of brain tissue. Such patients include those who have experienced a stroke, brain aneurysm, brain infection, brain **tumor**, brain bleeding or brain blood clot.

DETD . . . the antisense nucleic acids can be administered to the subject in vivo, for example, to a subject that has a **tumor** in a nerve tissue. In another embodiment, the antisense compositions can be transformed into a cell that is contacted ex. . .

DETD . . . is operatively linked to a gene encoding a protein that enhances formation of a neuromuscular synapse, for example, rapsyn, utrophin, **dystroglycan**, MuSK, **laminin** .beta.-2, and agrin and similar components of synapses in nerve and muscle tissue.

DETD . . . making available purified and recombinant nucleic acid constructs which are fusions of the .epsilon.-promoter NRE to suitable reporter genes, provides **assays** which can be used to screen for drugs which are either agonists or antagonists. By mutagenesis, and by structural surveys. . .

DETD . . . Whether a change in the amino acid sequence of a peptide results in a functional protein homolog can be readily **determined** by assessing the ability of the variant peptide to effect an appropriate response in cells in a fashion similar to. . .

DETD . . . clinical setting with a particular symptom or symptoms

suggesting treatment by the compositions or methods of the invention. A patient's **diagnosis** can alter during the course of disease progression, such as development of further disease symptoms, or remission of the disease,. . .

DETD . . . by PTPase and activated Ras or equivalent proteins within the same signal transduction pathway . Equivalence of function can be **determined** without undue experimentation by the methods provided herein.

DETD . . . transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (e.g. **antibody** conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the. . .

DETD . . . and unique restriction sites for insertion of genes downstream of a variant LTR from the retroviral mutant PCMV (PCC4 embryonal **carcinoma** cell-passaged myeloproliferative sarcoma virus).

DETD . . . publications WO93/25234, WO94/06920, and WO94/11524). For instance, strategies for the modification of the infection spectrum of retroviral vectors include: coupling **antibodies** specific for stem cell surface antigens to the viral env protein (Roux et al. (1989) PNAS 86:9079-9083; Julan et al.. . . variety (e.g. lactose to convert the env protein to an asialoglycoprotein), as well as by generating fusion proteins (e.g. single-chain **antibody**/env fusion proteins).

DETD In yet another illustrative embodiment, the gene delivery system comprises an **antibody** or cell surface ligand which is cross-linked with a gene binding agent such as polylysine (see, for example, PCT publications. . .

DETD . . . modified by nucleotide substitution, addition or deletion while the desired functionality is maintained. Furthermore, the methods invented herein provide numerous **assays** that can be performed to confirm that a functional equivalent or homolog of an .epsilon.-subunit regulatory element, is capable of regulating synapse-specific expression of a gene to which it is operably linked. Examples of these **assays** are set forth in the Example herein or are known in the art.

DETD Numerous **assays** can be performed to confirm that a promoter or a regulatory element is capable of controlling the expression of an. . . gene for luciferase. Preparation of these constructs and introduction into cells can be performed according to standard techniques. For this **assay**, it is desirable to transfect cell lines with the construct, that is cells or cell lines in which expression is. . .

DETD Another **assay** for **determining** the activity of a regulatory element is transfection of the regulatory element operably linked to a reporter gene into a cell line that is capable of differentiating in vivo. In this **assay**, the cell differentiates into a cell in which expression of the exogenous gene is desired. For example, embryonic muscle progenitors. . .

DETD An **assay** used to confirm cell studies of expression is to **determine** nuclear and cell response to plasmids injected to the muscles of an animal in vivo. Expression of appropriate linked reporter genes,. and subcellular location of said expression, can be **determined** at the nuclear level after sacrifice of the animal.

DETD Alternatively, the transcriptional activity of a regulatory element can be **assayed** by preparing transgenic mice containing the specific element as the transgene. Transgenic mice can be prepared according to methods known. . .

DETD One of ordinary skill in the art can **determine** and prescribe the effective amount of the pharmaceutical composition required. For example, one could start doses of the known or. . .

DETD . . . post-transfection, cells were placed in DMEM supplemented with 0.5% FCS for 48 h prior to harvesting for luciferase and CAT **assays**.

DETD . . . 2% horse serum). Cells were treated 24 hours later with neuregulin (5 nM), incubated for 60 h, and harvested and **assayed** for luciferase and CAT.

DETD In Vivo Expression **Assays**

DETD The in vivo expression **assay** involving direct injection of DNA into muscle has been described previously (Walke, W. et al. (1996) J. Neurosci. 16, 3641-3651;. . .

DETD . . . subcloned into the pXP (Nordeen, S. K. (1988) BioTechniques 6, 454-457) vector for expression studies (FIG. 1). Deletion endpoints were **determined** by DNA sequencing. The pXP vector carries a luciferase reporter gene.

DETD . . . and BspE1 (blunted) sites of .epsilon.-2000 BSSK (Walke, W. et al. (1994) J. Biol. Chem. 269, 19447-19456). Deletion endpoints were **determined** by DNA sequencing, and the mutation was then subcloned into Sma1 and Xho1 sites of pXP2 for expression studies.

DETD **Analysis** of expression programmed by .epsilon.-promoter 5' and 3' deletion mutants was used to identify a PTPase and neuregulin responsive element.. . .

DETD . . . No. 3) in the rat nAChR .epsilon.-subunit gene contains nucleotides that are required for regulation by PTPase, neuregulin and Ras. **Assays** of in vivo expression show that this sequence also participates in extrajunctional suppression of the .epsilon.-subunit gene. Thus, this sequence. . .

DETD . . . site (N box) mutant was created in mutant .epsilon.-154. The effect of this mutation reduced expression below the limits of **detection** in in vivo injection **assays**. Thus 5' sequences both at and upstream of nucleotide -154 participate in extrajunctional expression. These results indicated that the mouse. . . L. M. et al. (1995) Develop. Biol. 172, 158-169). Inspection of rat .epsilon.-promoter sequences -69 to -55 that comprise sequence **determinants** for synapse-specific expression, identified two nucleotides that differ between rat and mouse (TAAACCTAGTCCGGA, SEQ ID No.:3, in rat compared to. . .

DETD . . . family is: C/T GGA A/T, where the term C/T indicates that both C and T are represented in the sequences **analyzed** to identify a consensus. In the present invention, the 15-bp NRE identified from the rat nAChR (-subunit promoter includes the. . .

DETD . . . by probing with radiolabeled primers carrying the mouse Ets-2 sequence. Six plaques identified in this manner were purified, and further **analyzed** by **determination** of the DNA sequence of the inserted DNA.

DETD Transcription modulated in rat muscle L6 cells at the 15 bp NRE was assessed using the (5000-luciferase construct by **assaying** luciferase activity under each experimental condition. A deletion mutation of the rat Ets-2 gene was constructed by standard techniques of. . .

=> d bib abs 4

L53 ANSWER 4 OF 14 USPATFULL
AN 2000:28108 USPATFULL
TI SH3 kinase domain associated protein, a signalling domain therein, nucleic acids encoding the protein and the domain, and **diagnostic** and therapeutic uses thereof
IN Sudol, Marius, New York, NY, United States
Bork, Peer, Heidelberg, Germany, Federal Republic of
Chen, Henry, New York, NY, United States
PA The Rockefeller University, New York, NY, United States (U.S. corporation)
The Max Delbrveck Center for Molecular Medicine, Berlin-Buch, Germany, Federal Republic of (non-U.S. corporation)
PI US 6034212 20000307
AI US 1995-476509 19950607 (8)
RLI Continuation-in-part of Ser. No. US 1994-348518, filed on 1 Dec 1994
DT Utility
FS Granted
EXNAM Primary Examiner: Priebe, Scott D.; Assistant Examiner: Nguyen, Dave Trong
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CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 21 Drawing Figure(s); 27 Drawing Page(s)
LN.CNT 3669
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to regulation and control of cellular processes by SH3-domain binding proteins, by putative signalling domains of such proteins, ligands of the signalling domain, and **diagnosis** and therapy based on the activity of such proteins, signalling domains, and ligands.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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TI SH3 kinase domain associated protein, a signalling domain therein, nucleic acids encoding the protein and the domain, and **diagnostic** and therapeutic uses thereof

AB . . . of cellular processes by SH3-domain binding proteins, by putative signalling domains of such proteins, ligands of the signalling domain, and **diagnosis** and therapy based on the activity of such proteins, signalling domains, and ligands.

GOVI . . . part with Grant No. CA51083 from the National Institutes of Health, and Grant Nos. CA45757 and CA01605 from the National **Cancer** Institute. Accordingly, the Government may have certain rights in the invention.

SUMM . . . of cellular processes by SH3-domain binding proteins, by putative signalling domains of such proteins, ligands of the signalling domain, and **diagnosis** and therapy based on the activity of such proteins, signalling domains, and ligands.

SUMM Our functional studies of the Yes proto-oncogene started with the generation of polyclonal **antibodies** directed to the bacterially expressed fusion protein corresponding to the unique and SH3 domains of Yes (Sudol & Hanafusa, 1986). Interestingly, the resulting **antibody** showed strong immunoreactivity with the SH3 domain and weaker reaction with the unique domain. Based on this observation we used the original anti-Yes IgG to generate polyclonal anti-idiotypic **antibodies** (Jerne, 1974) expecting a reagent that would mimic a conformation of the SH3 domain of Yes and would allow us. . .

SUMM . . . characterization and cDNA cloning of a novel protein that binds to the SH3 domain of the Yes proto-oncogene product. Anti-idiotypic **antibodies** were used to identify the protein and to clone its cDNA from an expression library. The presence of serine phosphorylation. . .

SUMM . . . an SH3 domain, binding to an approximately 40 kDa intracellular ligand, binding to a dystrophin-associated protein, regulation of binding of .beta.-**dystroglycan** to dystrophin, and modulation of intracellular signalling.

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SUMM In addition to proteins, the invention extends to an **antibody** that binds to the protein or polypeptide of the invention. Such **antibodies** may be polyclonal or **monoclonal**, and are intended to include single chain, Fv fragments, F(ab) fragments, chimeric **antibodies**, humanized **antibodies**, bacterially expressed **antibodies**, etc. In a specific embodiment, the **antibody** can inhibit the functional activity of the protein or polypeptide.

SUMM . . . discovered that the WW domain interacts with a proteinaceous ligand in the cytoplasm. This ligand has been identified by "Western" **analysis** (using labeled WW domain) as having an approximate molecular weight of 35-36 kDa. cDNAs encoding the ligand have also been. . .

SUMM . . . a method for identifying a ligand of a WW domain polypeptide, comprising contacting candidate ligands with the WW domain polypeptide, **detecting** binding of the WW domain polypeptide with a ligand; and **determining** the structure of the ligand. The invention naturally relates to the ligand identified by this method, and as characterized above.. . .

SUMM . . . cells transformed with candidate DNA believed to encode a ligand of the WW domain polypeptide with the WW domain polypeptide; **detecting** binding of the WW domain polypeptide with a ligand expressed by the transformed cells; selecting transformed cells in which binding of the WW domain polypeptide is **detected**; and **determining** the structure of a nucleic acid in the selected cells which corresponds to the transforming DNA which encodes the ligand.. . .

SUMM The proteins and polypeptides of the invention, and nucleic acids encoding the same, are useful for **diagnosis** and therapy of a

disease or disorder associated with a defect in intracellular signal transduction. For example, the invention relates. . .

SUMM . . . polypeptide, e.g., to decrease cellular activation associated with intracellular signalling. Such therapy may be important in the treatment of certain **cancers** and **tumors**. Inhibition can be achieved with neutralizing **antibodies**, by gene knockout, with antisense nucleic acids, and the use of small molecule antagonists (e.g., a competitive inhibitor such as. . .

DRWD . . . 2 were precipitated with preimmune sera; lanes 3 and 4 were precipitated with immune sera. (B) Lanes 5-9: Immune blot **analysis**. Immunoprecipitates with preimmune (lane 5) or anti-idiotypic immune (lane 6) serum, or total lysates of primary (lane 7), secondary (lane. . . I-labeled-protein A. Solid arrows indicate YAP65, and an open arrow shows 120 kDa protein. The 120 kDa protein was not **detected** on the immune blot. Molecular size markers are shown in kDa.

DRWD . . . the chicken YAP65 CDNA and the predicted protein product. The CDNA sequence of the original clone isolated with the anti-idiotypic **antibodies** is indicated with arrows. The sequence of a proline-rich motif implicated in the binding of YAP65 to the SH3 domain. . .

DRWD . . . P.sub.i]. One dimensional tryptic peptide mapping of YAP65 precipitated with IgG against TrpE-YAP65 fusion protein (7) or with anti-idiotypic **antibody** (8); lane 9, tryptic peptide map of the 120 kDa protein precipitated with anti TrpE-YAP65 or with anti-idiotypic **antibody** (lane 10). One dimensional phosphoamino acid **analysis** of YAP65 (lane 11) and 120 kDa protein (lane 12). O-origin of the sample application; P-Y, phosphotyrosine; P-T, phosphothreonine; P-S,. . .

DRWD FIG. 4. Northern blot **analysis** of YAP65 and Yes mRNAs. Five micrograms of polyA.sup.+ mRNA from telencephalon (1), or cerebellum (2), spleen (3), intestine (4),. . .

DRWD . . . Lysates of CEFs were immunoprecipitated with anti-Yes IgG (2) or with YAP65-Sepharose (4,5,6) and subjected to an immune complex kinase **assay**. Preimmune IgG--lane 1; Sepharose-4B--lane 3. Lane 5 is immunoprecipitation with YAP65-Sepharose in the presence of 2 .mu.M of GST-Yes-SH3 fusion. . . in vitro YAP65 interacts with the SH3 domain of Src. The doublet of bands observed in the results of kinase **assays**, lanes 4 and 5, is characteristic for Yes kinase (for discussion see Sudol & Hanafusa, 1986). (B) Western blot **analysis** of samples shown in (A). Proteins transferred to nitrocellulose were probed with anti-Yes IgG and .sup.128 I-labeled protein A. Open arrow indicates products of the in vitro kinase **assay**. Solid arrow indicates the Yes protein.

DRWD FIG. 9. Southern blot **analysis** of genomic DNA from nine eukaryotic species. Genomic DNA (4 .mu.g) was digested with EcoRI, resolved in 0.7% agarose gel,. . .

DRWD FIG. 10. Northern blot **analysis** of poly A.sup.+ RNA from sixteen different human tissues. Poly A.sup.+ RNAs (2 .mu.g each) from adult human tissues were. . .

DRWD YAP65 cDNA **detects** loci on human chromosomes 11 and 6. DNA (.about.10 .mu.g/lane) from human (lane 1), hamster-human hybrid 7300 with human chromosomes. . .

DRWD FIG. 18. Binding **assays** with WBP-1 and putative binding domain. Two independent clones of each GST fusion construct were chosen and induced for protein. . .

DRWD FIG. 19. Mutational **analysis** of PY motif. The residues comprising the PY motif were each changed to alanine. (A and B) Lanes: 1, GST;. . . (A) GST fusion proteins expressing each of these mutated PY motif along with the five invariant flanking residues were then **assayed** for binding activity to labelled GST-WW-YAP (arrow). (B) The amount of protein loaded in each well (2 mg) was equivalent. . .

DETD . . . the invention provides nucleic acids, particularly DNA molecules, encoding such proteins and polypeptides. In one aspect, the invention relates to **diagnosis** of diseases or disorders, employing the polypeptides and nucleic acids of the invention. The invention further relates to modulation of. . .

DETD . . . is capable of (i) serving as a substrate for proteolytic cleavage (e.g., a Factor Xa sequence); (ii) binding to an **antibody** specific for the fusion partner protein; (iii) binding

to a cognate receptor or a ligand; (iv) interacting ionically or hydrophobically with a chromatographic support; (v) catalyzing a reaction, i.e., enzymatic activity; or (vi) otherwise biologically active as **assayed** in vitro or in vivo.

DETD . . . in common among all of the proteins or polypeptides. In a specific embodiment, the consensus sequence can be defined by **determination** that putative consensus segments have a probability of less than 1 in 10.sup.6, and preferably less than 1 in 10.sup.7,. . .

DETD . . . approximately 40 kDa intracellular ligand (or inhibition thereof), binding to a dystrophin-associated protein (or inhibition thereof), regulation of binding of .beta.-**dystroglycan** to dystrophin, and modulation of intracellular signalling.

DETD . . . appropriate conditions of temperature and solution ionic strength (see Sambrook et al., supra). The conditions of temperature and ionic strength **determine** the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T.sub.m. . . hybridization with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide **determines** its specificity (see Sambrook et al., supra, 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid is at least. . .

DETD . . . vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are **determined** by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A. . .

DETD . . . and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels **detectable** above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with. . .

DETD . . . when it is capable of specifically interacting with an antigen recognition molecule of the immune system, such as an immunoglobulin (**antibody**) or T cell antigen receptor. An antigenic polypeptide contains at least about 5, and preferably at least about 10, amino acids. An antigenic portion of a molecule can be that portion that is immunodominant for **antibody** or T cell receptor recognition, or it can be a portion used to generate an **antibody** to the molecule by conjugating the antigenic portion to a carrier molecule for immunization. A molecule that is antigenic need. . .

DETD . . . directed to YAP proteins, polypeptides comprising or consisting primarily of the WW domain, to nucleic acids encoding such proteins, to **antibodies** reactive with the proteins, and to methods of use of the proteins, polypeptides, and acids.

DETD . . . based, in part, on the isolation and characterization of a unique Yes-associated protein from chicken, based on screening with anti-idiotypic **antibodies** generated against Yes SH3-specific polyclonal **antibodies**. With the chicken gene in hand, the human and murine orthologs (homologous genes in different species) were quickly recovered. Expression. . . prostate, testis, ovaries, and small intestine, and relatively lower levels in brain, liver, and spleen. No YAP mRNA expression was **detectable** in human peritoneal leukocytes, even with overexposure of the blot.

DETD . . . and nucleic acids encoding them; ligands of the WW domain, and genes inducing them; isolating genes and expressing recombinant proteins; **antibodies** to the proteins; antisense nucleic acids; **diagnostic** applications; and therapeutic applications.

DETD . . . the human dystrophin WW domain and human YAP WW domain) described above and specifically exemplified infra, can be used to **detect** the presence of a ligand to the WW domain. In this way, a 35-36 kDa protein has been identified as. . .

DETD Once partial cDNA clones are obtained from the expression library, the full length cDNA can be obtained, and the sequence **determined** and **analyzed**. From this information, a putative amino acid sequence can be deduced, and characteristics about the gene and the polypeptide can. . .

DETD Alternatively, the presence of the gene may be **detected** by **assays** based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA

clones which. . . properties as known for the gene produce, e.g., YAP, the WW domain, or the WW domain ligand. For example, the **antibodies** of the instant invention can conveniently be used to screen for homologs of YAP from other sources, preferably human.

DETD . . . (d) expression of inserted sequences. In the first approach, the nucleic acids can be amplified by PCR to provide for **detection** of the amplified product. In the second approach, the presence of a foreign gene inserted in an expression vector can be **detected** by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted gene. In the third approach, the. . . identified by the absence of the marker gene function. In the fourth approach, recombinant expression vectors can be identified by **assaying** for the activity of the gene product expressed by the recombinant, provided that the expressed protein assumes a functionally active conformation. Such **assays** can be based, for example, on the physical or functional properties of the gene product in in vitro **assay** systems, e.g., tyrosine phosphorylation, or alternatively binding with **antibody**.

DETD . . . (Smith and Johnson, 1988, Gene 67:31-40). The ligation mixture can then be transformed into E. coli and the clones obtained **analyzed** by restriction digestion and DNA sequencing. Products of resulting plasmids can be purified over glutathione-SEPHAROSE resin and eluted with free. . .

DETD . . . After 72 hours, cells can be lysed by Dounce homogenization in TNE buffer, and protein products purified by gel filtration, **antibody** affinity chromatography, or a combination of chromatography steps.

DETD Once a recombinant which expresses the gene sequence is identified, the recombinant product can be **analyzed**. This is achieved by **assays** based on the physical or functional properties of the product, including radioactive labelling of the product followed by **analysis** by gel electrophoresis, **immunoassay**, etc.

DETD For example, the ability of the expressed protein, or a fragment thereof, to function in an **assay**, can be **determined**.

DETD The structure of a YAP protein, a WW domain polypeptide, or a WW domain ligand of the invention can be **analyzed** by various methods known in the art. Preferably, the structure of the various domains, particularly the domain, is **analyzed**. Structural **analysis** can be performed by identifying sequence similarity with other known proteins, as was performed in identifying the WW domain. The. . .

DETD The protein sequence can be further characterized by a hydrophilicity **analysis** (e.g., Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824). A hydrophilicity profile can be used to identify the. . .

DETD Secondary structural **analysis** (e.g., Chou and Fasman, 1974, Biochemistry 13:222) can also be done, to identify regions of a protein or polypeptide that. . .

DETD By providing an abundant source of recombinant proteins and polypeptides, the present invention enables quantitative structural **determination** of the protein, or domains thereof. In particular, enough material is provided for nuclear magnetic resonance (NMR), infrared (IR), Raman, and ultraviolet (UV), especially circular dichroism (CD), spectroscopic **analysis**. In particular NMR provides very powerful structural **analysis** of molecules in solution, which more closely approximates their native environment (Marion et al., 1983, Biochem. Biophys. Res. Comm. 113:967-974;. . . al., 1985, J. Magn. Reson. 65:355-360; Kimura et al., 1980, Proc. Natl. Acad. Sci. U.S.A. 77:1681-1685). Other methods of structural **analysis** can also be employed. These include but are not limited to X-ray crystallography (Engstom, A., 1974, Biochem. Exp. Biol. 11:7-13).

DETD In a specific embodiment, the crystal structure of human YAP and human dystrophin are being obtained and compared, to **determine** the molecular consequences of the observed similarity between these proteins, particularly at the level of the WW domain.

DETD . . . the binding reaction can be studied. Similarly, co-crystals of the WW domain and the WW domain ligand can be prepared. **Analysis** of co-crystals provides detailed information about binding, which in turn allows for rational design of ligand agonists and antagonists.

Computer. . .

DETD **Antibodies**

DETD . . . . fragments or other derivatives or analogs thereof, or cells expressing the foregoing may be used as an immunogen to generate **antibodies** which recognize the cognate protein or polypeptide. Such **antibodies** include but are not limited to polyclonal, **monoclonal**, chimeric, single chain, Fab fragments, and an Fab expression library. In another embodiment, infra, anti-idiotype **antibodies** can be generated to a binding partner of the protein or polypeptide, for example to anti-Yes **antibodies**, in order to obtain **antibodies** reactive, in this instance, with YAP. Moreover, it was a surprising result that such **antibodies** could in fact be obtained.

DETD Various procedures known in the art may be used for the production of polyclonal **antibodies** to a recombinant or derivative or analog thereof. For the production of **antibody**, various host animals can be immunized by injection with the recombinant, or a derivative (e.g., fragment) thereof, including but not. . .

DETD For preparation of **monoclonal antibodies** directed toward an or analog thereof, any technique which provides for the production of **antibody** molecules by continuous cell lines in culture may be used. These include but are not limited to the hybridoma technique. . . technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human **monoclonal antibodies** (Cole et al., 1985, in **Monoclonal Antibodies** and **Cancer** Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, **monoclonal antibodies** can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human **antibodies** may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, in **Monoclonal Antibodies** and **Cancer** Therapy, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric **antibodies** " (Morrison et al., 1984, J. Bacteriol. 159-870; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse **antibody** molecule specific for a .lambda. together with genes from a human **antibody** molecule of appropriate biological activity can be used; such **antibodies** are within the scope of this invention. Such human or humanized chimeric **antibodies** are preferred for use in therapy (described infra), since the human or humanized **antibodies** are much less likely than xenogenic **antibodies** to induce an immune response, in particular an allergic response, themselves.

DETD According to the invention, techniques described for the production of single chain **antibodies** (U.S. Pat. No. 4,946,778) can be adapted to produce specific single chain **antibodies**. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of **monoclonal** Fab fragments with the desired specificity.

DETD **Antibody** fragments which contain the idiotype of the **antibody** molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragment which can be produced by pepsin digestion of the **antibody** molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragment; and the Fab fragments which can be generated by treating the **antibody** molecule with papain and a reducing agent.

DETD In the production of **antibodies**, screening for the desired **antibody** can be accomplished by techniques known in the art, e.g., **radioimmunoassay**, ELISA (enzyme-linked immunosorbant **assay**), "sandwich" **immunoassays**, immunoradiometric **assays**, gel diffusion precipitin reactions, immunodiffusion **assays**, in situ **immunoassays** (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination **assays** (e.g., gel agglutination **assays**, hemagglutination **assays**),

complement fixation **assays**, immunofluorescence **assays** , protein A **assays**, and immunoelectrophoresis **assays** , etc. In one embodiment, **antibody** binding is **detected** by **detecting** a label on the primary **antibody**. In another embodiment, the primary **antibody** is **detected** by **detecting** binding of a secondary **antibody** or reagent to the primary **antibody**. In a further embodiment, the secondary **antibody** is labeled. Many means are known in the art for **detecting** binding in an **immunoassay** and are within the scope of the present invention. For example, to select **antibodies** which recognize a specific epitope, one may **assay** generated hybridomas for a product which binds to a fragment containing such epitope. For selection of an **antibody** specific to an YAP, WW domain, or WW domain ligand from a particular species of animal, one can select on. . .

DETD The foregoing **antibodies** can be used in methods known in the art relating to the localization and activity of their binding partners, e.g., for Western blotting, imaging, **measuring** levels thereof in appropriate physiological samples, etc.

DETD In a specific embodiment, **antibodies** that agonize or antagonize the activity of can be generated. Such **antibodies** can be tested using the **assays** described infra for identifying ligands.

DETD . . . expressed after transfection or transformation of the cells. According, the present invention contemplates identifying specific ligands of using various screening **assays** known in the art.

DETD . . . an initial clue as the inhibitors or antagonists of the protein. Identification and screening of antagonists is further facilitated by **determining** structural features of the protein, e.g., using X-ray crystallography, neutron diffraction, nuclear magnetic resonance spectrometry, and other techniques for structure **determination**. These techniques provide for the rational design or identification of inhibitors and antagonists.

DETD **Diagnostic** and Therapeutic Compositions and Methods

DETD . . . and the ability to modulate activity of such proteins and polypeptides of the invention, can be very important for the **diagnosis** and treatment of diseases of disorders, particularly cellular transformations that lead to **cancer**, and to disorders such as muscular dystrophy.

DETD Thus, the nucleic acid probes (enzyme or radio-labeled nucleotides) or **antibodies** of the invention can be used to **detect** expression, and **measure** the level of expression, of a YAP protein, or a protein carrying a WW consensus sequence of the invention in selected tissues. For example, the presence or absence of expression of YAP in **cancer** cells obtained in a tissue biopsy can be important in evaluating whether the normal cellular control machinery are operating. Similarly,. . .

DETD In another embodiment, the level of Yes and other SH3-containing proteins can be evaluated by **detecting** the level of binding of YAP protein to the sample being **assayed**. In a further aspect, signal transduction can be evaluated by **detecting** the level of phosphorylation of the YAP protein in cells in vivo.

DETD In a further embodiment, **antibodies** generated to YAP, the WW domain or domains, or to the WW domain ligand can be used to evaluate the presence or level of activity of the proteins or polypeptides. **Immunoassays** can be performed by any of the standard techniques described above. The presence of low levels of YAP or particular. . .

DETD . . . to the identification of a novel gene and its deduced protein product. This protein was isolated by binding to anti-idiotype **antibodies** against the amino terminal domain of Yes, a member of the Src family of protein-tyrosine kinases involved in signaling. The. . .

DETD Cells and **Antibodies**

DETD . . . a portion of bacterially express Yes protein that contains its entire unique and SH3 domains (Sudol & Hanafusa, 1986). Anti-idiotypic **antibodies** (Jerne, 1974) were raised in rabbits following a published protocol (Strosberg, 1989). Two rabbits were injected with 500 .mu.g affinity. . . half months after the initial injection and continued in 2 week intervals. After the second boost the serum showed immunoreactivity. **Antibodies** against YAP65 were generated in

rabbits against a portion of the YAP65 sequence (nucleotides 381-1298) expressed in bacteria using the. . . of the betagalactosidase protein in the original lambda gt11 clone (1 kb long clone) indicated the reading frame of YAP65. **Antibodies** against the human GAP protein that recognize also the chicken GAP protein on Western blots were purchased from UBI (Lake. . .

DETD **Immunoassays**

DETD Cell lysates were prepared in 150 mM NaCl RIPA buffer with protease inhibitors (Sudol & Hanafusa, 1986). The autophosphorylation kinase **assay** and Western blot **analyses** were as previously described (Sudol & Hanafusa).

DETD . . . protein, from CEFs metabolically labeled with [.sup.35 S]methionine (FIG. 1, lanes 3 and 4). The 65 kDa protein was also **detected** by immune blot **analysis** in total lysates of CEFs from various passages (FIG. 1, lanes 6-9). The even intensity of the 65 kDa band. . .

DETD High levels of Yes expression in cerebellum (Sudol et al., 1989) and the **detection** of YAP65 in cerebellum by immune blot (data not shown) pointed to a source of RNA for the isolation of. . .

DETD . . . by anti-idiotypic sera, we have expressed a part of the cDNA in bacteria using a TrpE operon based vector. Polyclonal **antibodies** generated in rabbits against the Trp-E-YAP65 fusion protein precipitated from CEFs a 65 kD protein that comigrated with YAP65 identified by anti-idiotypic sera (FIGS. 3, lanes 7 and 8. By the same method, we have also **determined** that the 120 kDa protein precipitated with anti-idiotypic **antibodies** and with **antibodies** generated against the bacterially expressed YAP65 protein are identical (FIG. 3, lanes 9 and 10). By the criterion of the. . .

DETD On Northern blots, the YAP65 cDNA **detected** a single 4.2 kb transcript expressed ubiquitously in various chicken tissues including brain (telencephalon, cerebellum), heart, spleen, intestine, liver, kidney. . .

DETD We **assayed** for binding between the bacterially expressed fusion proteins of YAP65 and Yes. As shown in FIG. 5a, lanes 1 and. . . show any binding to TrpE-YAP65 (data not shown). To show binding specificity, we used cold GST-YES-SH3 protein in a competition **assay** (FIG. 5a, lane 4). In order to evaluate the involvement of the proposed proline-rich motif of YAP65 in binding to. . .

DETD . . . fusion proteins of Nck, Crk, Src, Abl and GAP with radioactively labeled Trp-E-YAP65 protein. The same amount of protein was **analyzed** in a membrane binding **assay**; TRPE-YAP-65 bound the strongest to Nck and Yes followed by Crk, and Src. Binding of TrpE-YAP65 to the GST-SH3 domains. . .

DETD To document direct interaction between YAP65 and Yes we attempted to coprecipitate Yes with YAP65 **antibodies** and YAP65 with Yes **antibodies**. The results were negative. However, when we partially purified YAP65 protein from CEFs and coupled it covalently to Sepharose beads,. . .

DETD Using polyclonal **antibodies** raised in rabbits against affinity purified polyclonal **antibodies** recognizing the unique and SH3 domain of the Yes protein, we **detected** a 65 kDa protein (YAP65) that form a complex with th Yes proto-oncogene product in in vitro **assays**. With thus generated **antibodies**, we cloned the YAP65 CDNA from an expression library. By a number of criteria, we showed that YAP65 interacts specifically. . .

DETD The following aspects of the work deserve brief comment: (i) the use of polyclonal **antibodies** in the generation of anti-idiotypic **antibodies**; (ii) the identity of the 120 kDa protein that is found in YAP65 immunoprecipitates; (iii) the hallmarks and subtle features. . .

DETD The decision to generate polyclonal anti-idiotypic **antibodies** against polyclonal **antibodies**, rather than to use **monoclonal antibodies** as antigens, stemmed from two observations. (i) The primary anti-Yes serum was generated against a portion of the Yes protein. . . both regions were represented in the antigen in equivalent molar amounts (Sudol, unpublished). (ii) Mapping of binding domains for the **monoclonal antibodies** generated against another closely related kinase, Src, provided suggestive evidence on the `immunodominance` of epitopes within the SH3 domain (Parsons et al., 1986). Based on these two observations, we

argued that by using polyclonal **antibodies** (first **antibody**, anti-Yes) directed to the apparently dominant epitope(s) (Yes SH3), we may obtain anti-idiotypic **antibodies** (Jerne, 1974) that would mimic the Yes SH3 domain and bind to its putative cellular targets.

DETD In addition to YAP65, both the anti-idiotypic **antibodies** and **antibodies** generated against bacterially expressed YAP65 cDNA recognized another protein of 120 kDa. The peptide mapping **analysis** showed that the 120 kDa protein is not a precursor of YAP65. Although the 120 kDa protein was not **detected** on Western blots, we cannot presently **determine** whether it shares epitopes with YAP65 or whether it is a YAP65 binding protein. The former possibility seems likely since. . . is a chimeric clone or whether it corresponds to a novel CDNA. The 120 kDa protein is not recognized by **antibodies** that recognize the human GAP protein (data not shown). The YAP65 cDNA contains one long open reading frame that ends. . .

DETD . . . purified YAP65 coupled to Sepharose beads (FIG. 6). However, we were not able to coprecipitate Yes and YAP65 using available **antibodies**. It is likely that these **antibodies** prevent complex formation by binding at or near the domains involved in the interaction.

DETD . . . the proline-rich domain of YAP65 (PLAP peptide) was also able to block the recognition of YAP65 by the original anti-idiotypic **antibody** (not shown). In view of the fact that a large concentration (200 .mu.M) of the PLAP peptide was required to. . .

DETD Sudol, M. (1993). The Molecular Basis of **Cancer**, Neel, B. & Kumar, R. (eds). Futura: N.Y., pp. 203-224.

DETD . . . pEXlox-MYAP6 (2.3 kb Eco RI-Hind III insert) and pEXlox-MYAP20 (Eco RI-Hind III insert). Both strands of the cDNA clones were **analyzed** by direct sequence **analysis** using the Sanger method.

DETD Southern and Northern Blot **Analysis**--southern blot on genomic DNA from nine eukaryotic species was performed using the same conditions as for cDNA library screening. DNA. . . a specific activity of approximately 2.times.108 cpm/.mu.g and were used as a probe for Southern (HYAP probe) and for Northern **analysis** (HYAP probe first, and after striping the probe for beta actin). Poly A.sup.+ RNAs were isolated from 16 different human tissues. The age and sex of tissue donors varied but all tissues, as far as could be **determined**, were free of disease (Clontech Lab, Inc. Palo Alto, Calif.). The RNA (2 .mu.g per lane) were run on a. . .

DETD . . . et al., 1986). Hybrid DNAs were tested for presence of YAP65 specific human Sst I and Pst I restriction fragments **detected** by radiolabeled YAP65 probe using standard Southern hybridization methods.

DETD . . . et al., 1993). Probes were prepared by nick translation using biotin-labeled 11-dUTP (Bionick kit, BRL). Hybridization of biotin-labeled probes was **detected** with fluorescein isothiocyanate-conjugated avidin. Metaphase chromosomes were identified by Hoechst-33528 staining and UV irradiation (365 nm), followed by 4', g-diamidino-2-phenylindole. . .

DETD Computer-Aided **Analysis** of Protein Sequences--Searches of sequence homology were performed through the FASTA and FASTP programs in GenBank. The secondary structures of. . .

DETD . . . Of 13 positive clones, two (HYAP5 and HYAP6) with the longest inserts (approximately 3 and 5 kb long, respectively) were **analyzed** further. Initial **analysis** of the DNA sequence showed that HYAP5 cDNA is included with the HYAP6 clone. The result of direct sequence **analysis** of both strands of the HYAP6 cDNA is shown in FIG. 7. The longest open reading frame predicted a protein. .

DETD We have subjected this sequence to a more extensive **analysis** and found that the motif shares significant sequence and putative structure similarities with sequences found in various regulatory and signalling. . .

DETD . . . with Various Eukaryotic DNAs--A high degree of sequence similarity between HYAP, MYAP and chicken YAP was confirmed by Southern blot **analysis** of the genomic DNAs digested with EcoRI enzyme (FIG. 9). Genomic DNA from other higher eukaryotes also showed

hybridization with the HYAP radioactive probe. However, no specific signal was **detected** in yeast Saccharomyces cerevisiae.

DETD Northern Blot **Analysis**--A major band of approximately 5 kb was **detected** in various human tissues. In addition a band migrating below the 2.4 kb mark was also **detected** in some of the tissues (see FIG. 10, lanes K, M and O for example). The expression of HYAP mRNA. . . . of the message were found in the brain, liver and spleen (FIG. 10, lanes B, E, I). We could not **detect** HYAP mRNA in the preparation of human peritoneal blood leukocytes even if the blot was overexposed (FIG. 10, lane P).

DETD Chromosomal localization--The HYAP cDNA **detected** two loci, one on chromosome 11 (11q13) and another on chromosome 6 (6q23-qter). When human DNA was digested with Sst. . . . radioactive HYAP cDNA, two strongly hybridizing bands one of 16 kbp and another migrating above 23 the kbp mark were **detected** (not shown). In addition, we also observed less strongly hybridizing bands. In the same **analysis**, rodent DNA digested with Sst I and probed with HYAP cDNA showed fainter bands distinguishable from the HYAP specific fragments. . . . two strongly hybridizing bands segregated independently and thus were on different chromosomes (not shown). The results of the more extensive **analysis** of the rodent-human hybrid panel are summarized in FIG. 11A. These data illustrate that one HYAP specific locus maps to. . .

DETD . . . data, fluorescent in situ hybridization (FISH) with the HYAP65 cDNA probe to normal human metaphases was performed. Using FISH we **detected** 51 signals at 11q13 on 27 metaphases and only 12 signals on the q terminal 1/3 of chromosome 6. The. . .

DETD . . . the BCL1 major breakpoint region, possibly within the chromosomal region which is amplified in a significant fraction of human mammary **carcinomas**, a panel of 17 mammary **carcinoma** cell line DNAs was tested for evidence of amplification of the HYAP65 gene. Four of these DNAs had shown amplification. . . not shown}. Thus, the HYAP65 gene is most likely centromeric to the chromosome region commonly amplified at 11q13 in mammary **carcinomas**.

DETD . . . J., Druck, T., Croce, C. M. and Huebner, K. Characterization of bone marrow derived closed circular DNA clones. Genes, Chrom. **Cancer** 7:15-27, 1993.

DETD . . . molecule; iii) a cysteine-rich calcium-binding domain; and iv) a C-terminal globular domain (Ahn and Kunkel, 1993, supra) (FIG. 12). Molecular **analysis** of the central rod-like portion of human dystrophin revealed two interruptions of the spectrin repeats and two flanking segments which. . .

DETD . . . the many dystrophin-associated proteins (Tinsley et al., 1994, Proc. National Acad. Sci USA 91:8307-13). It is closely located to the .beta.-**dystroglycan** binding site and may regulate the formation of this complex.

DETD . . . kB, the other is 0.5 kB. Preliminary and partial sequence data suggest that these clones encode two novel proteins. Sequence **analysis** indicates that they are not related to each other, and there is no significant degree of sequence similarity with any. . .

DETD . . . sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the nucleic acid sequence of the construct was confirmed by direct sequence **analysis** using the Sanger method (Sanger et al, (1977) Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467). Fusion proteins of the cloned WBP-1. . .

DETD . . . into the pEXlox vector as previously described (Palazzolo et al (1990) Gene 88:25-36). Both strands of the cDNA clones were **analyzed** by direct sequence **analysis** using the Sanger method (Sanger et al, 1977).

DETD Northern Blot **Analysis**

DETD . . . shown that YAP is present at high levels in lung, ovary, cerebellum, and skeletal muscle, thus increasing the likelihood of **detecting** the cognate ligand(s) in those organs as well (Sudol et al, 1995). Western ligand blot **analysis** revealed a band of approximately 38 kDa in size in lung, ovary, and cerebellum and an additional 34 kDa band. . .

DETD Northern Blot **Analysis** of the Ligands

DETD Binding **Assay** of Cloned Ligands

DETD . . . particular clone, as opposed to the three PY motifs in WBP-2, which may not all be functional. Western ligand blot **analysis** probed with .sup.32 P-labelled GST-WW-YAP showed binding to the

GST-ligand fusion proteins but not to GST alone (FIG. 18A). The. . . binding of GST-WW-YAP to GST-GTPPPPYTVG (SEQ ID NO:30) is reduced 50% from maximum binding (i.e., without any competing peptide) as **measured** by densitometry (data not shown).

DETD . . . GST fusion proteins, as previously described (Knudsen et al (1995) EMBO J., in press). Binding to WW-YAP domain was then **assayed** by probing blots of the mutant ligand proteins with .sup.32 P-labelled GST-WW-YAP (FIG. 19A). Binding was virtually abolished in the. . .

DETD . . . molecule that has been implicated in a specific disease phenotype (Duchenne's and Becker's muscular dystrophy), and other genetic approaches to **analyze** the WW domain of the yeast protein Rsp-5 should provide useful biological correlates.

=> d bib abs 5

L53 ANSWER 5 OF 14 USPATFULL
AN 2000:15516 USPATFULL
TI SH3 kinase domain associated protein, a signalling domain therein, nucleic acids encoding the protein and the domain, and **diagnostic** and therapeutic uses thereof
IN Sudol, Marius, New York, NY, United States
Bork, Peer, Heidelberg, Germany, Federal Republic of
Chen, Henry, New York, NY, United States
PA The Rockfeller University, New Yoek, NY, United States (U.S. corporation)
PI US 6022740 20000208
AI US 1994-348518 19941201 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Campell, Bruce R.; Assistant Examiner: Nguyen, Dave Trong
LREP Klauber & Jackson
CLMN Number of Claims: 33
ECL Exemplary Claim: 1
DRWN 20 Drawing Figure(s); 21 Drawing Page(s)
LN.CNT 3109
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to regulation and control of cellular processes by SH3-domain binding proteins, by putative signalling domains of such proteins, ligands of the signalling domain, and **diagnosis** and therapy based on the activity of such proteins, signalling domains, and ligands.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d bib abs 6

L53 ANSWER 6 OF 14 USPATFULL
AN 2000:1980 USPATFULL
TI Identification and isolation of novel polypeptides having WW domains and methods of using same
IN Pirozzi, Gregorio, East Windsor, NJ, United States
Kay, Brian K., Chapel Hill, NC, United States
Fowlkes, Dana M., Chapel Hill, NC, United States
PA University of North Carolina at Chapel Hill, Chapel Hill, NC, United States (U.S. corporation)
Cytogen Corp., Princeton, NJ, United States (U.S. corporation)
PI US 6011137 20000104
AI US 1996-630916 19960403 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Eyler, Yvonne
LREP Morgan & Finnegan, LLP
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 23 Drawing Figure(s); 20 Drawing Page(s)
LN.CNT 4094
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Novel polypeptides having WW domains of interest are described, along with DNA sequences that encode the same. A method of identifying these polypeptides by means of a sequence-independent (that is, independent of the primary sequence of the polypeptide sought), recognition unit-based functional screen is also disclosed. Various applications of the method and of the polypeptides identified are described, including their use in **assay** kits for drug discovery, modification, and refinement.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic 6

L53 ANSWER 6 OF 14 USPATFULL
AB . . . screen is also disclosed. Various applications of the method and of the polypeptides identified are described, including their use in **assay** kits for drug discovery, modification, and refinement.
SUMM 5.4. **ASSAYS** FOR THE DISCOVERY OF POTENTIAL DRUG CANDIDATES AND DISCOVERING THE SPECIFICITY THEREOF
SUMM 5.10. **ANTIBODIES** TO POLYPEPTIDES COMPRISING A WW DOMAIN
SUMM 6.5. BIOTINYLATED PEPTIDE **DETECTION** USING TYRAMIDE AMPLIFICATION SYSTEM
SUMM . . . acid SH3 region of Abl and isolated two clones that produced proteins capable of specifically binding the Abl SH3 domain. **Analysis** of one of the clones uncovered the region of the encoded protein responsible for binding to the SH3 domain. This. . .
SUMM . . . shown to be involved in the phenomenon of programmed cell death or apoptosis (Itoh et al., 1991, Cell 66:233-243). The **tumor** necrosis factor receptor 1 (TNFR-1) is also a member of this class (Baglioni, C., 1992, "The Molecules and Their Emerging Roles in Medicine," in **Tumor** Necrosis Factors, B. Beutler, ed. (New York: Raven Press). Itoh, N. and Nagata, S., 1993, J. Biol. Chem. 268:10932-10937 have. . .
SUMM where X represents any amino acid and bold letters represent highly conserved amino acids. Andre and Springaells **analysis** of WW domains led them to conclude that WW domains lack .alpha.-helical content, instead possessing a central .beta.-strand region flanked. .
.
SUMM . . . Ca.sup.2+ binding region, contains a WW domain. This WW domain is in an area that has been shown to bind .beta.-**dystroglycan**. This suggests that WW domains may be involved in protein--protein interactions (Bork and Sudol, 1994, Trends in Biochem. Sci. 19:531-533).
SUMM . . . an iterative process by which recognition units for WW domains identified in a first round of screening are used to **detect** WW domain-containing proteins in successive expression library screens.
SUMM The present invention also provides methods for identifying potential

new drug candidates (and potential lead compounds) and **determining** the specificities thereof. For example, knowing that a polypeptide with a WW domain and a recognition unit, e.g., a binding. . . on the polypeptide-recognition unit interaction, e.g., either as an agonist or as an antagonist (inhibitor) of the interaction. With this **assay**, then, one can screen a collection of candidate "drugs" for the one exhibiting the most desired characteristic, e.g., the most. . .

SUMM In addition, the present invention also provides certain **assay** kits and methods of using these **assay** kits for screening drug candidates. In a particular aspect of the present invention, the **assay** kit comprises: (a) a polypeptide containing a WW domain; and (b) a recognition unit having a selective affinity for the polypeptide. Yet another **assay** kit may comprise a plurality of polypeptides, each polypeptide containing a WW domain, preferably of a different sequence, and at. . .

DETD . . . from cDNA libraries. Generally, an appropriate cDNA library is screened with a probe that is either an oligonucleotide or an **antibody**. In either case, the probe must be specific enough for the gene that is to be identified to pick that. . .

DETD . . . protein products that might be encoded by the cDNA clones. If the probe used in prior art methods is an **antibody**, then it is necessary to build the cDNA library into a suitable expression vector. For a comprehensive discussion of the art of identifying genes from cDNA libraries, see Sambrook, Fritsch, and Maniatis, "Construction and **Analysis** of cDNA Libraries," Chapter 8 in Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, 1989. See also Sambrook, Fritsch, and Maniatis, "Screening Expression Libraries with **Antibodies** and Oligonucleotides," Chapter 12 in Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, 1989.

DETD . . . are used in prior art methods, the probe is virtually always a nucleic acid probe. See Sambrook, Fritsch, and Maniatis, " **Analysis** and Cloning of Eukaryotic Genomic DNA," Chapter 9 in Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press,. . .

DETD . . . an iterative process by which recognition units for WW domains identified in the first round of screening are used to **detect** WW domain-containing proteins in successive expression library screens (see FIGS. 2 and 6B). This strategy enables one to search "sequence. . .

DETD (b) **determining** at least part of the amino acid sequences of said peptides;

DETD (c) **determining** a consensus sequence based upon the **determined** amino acid sequences of said peptides; and

DETD (b) **determining** at least part of the amino acid sequence of said first peptide;

DETD The present invention also provides **antibodies** to a polypeptide having an amino acid sequence selected from the group consisting of: SEQ ID NOs: 30-37, and 38.

DETD The present invention also provides **antibodies** to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 46, 48, and. . .

DETD . . . be "probed" by the recognition unit, optionally in the presence of an inducer should one be required for expression, to **determine** if any selective affinity interaction takes place between the recognition unit and the individual clone. Prior to contacting the recognition. . .

DETD . . . equivalent thereof. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the recognition unit can be **determined** directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be **determined** more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence.

DETD If the amino acid sequence is to be **determined** from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

DETD . . . any unbound recognition unit from a mixture of the recognition unit and the plurality of polypeptides prior to attempting to **determine** or to **detect** the presence of a selective affinity interaction (i.e., the presence of a recognition unit that

remains bound after the washing. . .

DETD . . . when screening a library. High specificity is exhibited, e.g., by affinity purified polyclonal antisera which, in general, are very specific. **Monoclonal antibodies** are also very specific. Small peptides in monovalent form, on the other hand, generally give very weak, non-specific signals when. . .

DETD . . . recognition units in the form of small peptides, in multivalent form, have a specificity midway between the high specificity of **antibodies** and the low/non-specificity of monovalent peptides. Multivalency of the recognition unit of at least two, in a recognition unit complex. . .

DETD . . . serum albumin (BSA), keyhole limpet hemocyanin (KLH) by use of known cross-linking reagents. Such cross-linked peptide recognition units may be **detected** by, e.g., an **antibody** to the carrier protein or **detection** of the enzymatic activity of the carrier protein.

DETD The present invention is also directed to an **assay** kit which can be useful in the screening of drug candidates. In a particular embodiment of the present invention, an **assay** kit is contemplated which comprises in one or more containers (a) a polypeptide containing a WW domain; and (b) a recognition unit having a selective affinity for the polypeptide. The kit optionally further comprises a **detection** means for **determining** the presence of a polypeptide-recognition unit interaction or the absence thereof.

DETD . . . the polypeptide or the recognition unit. The polypeptide or, preferably, the recognition unit is immobilized on a solid support. The **detection** means employed to **detect** the label will depend on the nature of the label and can be any known in the art, e.g., film to **detect** a radionuclide; an enzyme substrate that gives rise to a **detectable** signal to **detect** the presence of an enzyme; **antibody** to **detect** the presence of an epitope, etc.

DETD A further embodiment of the **assay** kit of the present invention includes the use of a plurality of polypeptides, each polypeptide containing a WW domain. The **assay** kit further comprises at least one recognition unit having a selective affinity for each of the plurality of polypeptides and a **detection** means for **determining** the presence of a polypeptide-recognition unit interaction or the absence thereof.

DETD In the above **assay** kit, the polypeptide may comprise an amino acid sequence selected from the group consisting of SEQ ID NOs:12-28 and 29.. . .

DETD In other embodiments of the above-described **assay** kit, the recognition unit may be a peptide. The recognition unit may be labeled with e.g., an enzyme, an epitope,. . .

DETD The present invention also provides an **assay** kit comprising in one or more containers:

DETD The kits of the present invention may be used in the methods for identifying new drug candidates and **determining** the specificities thereof that are described in Section 5.4.

DETD 5.4. **ASSAYS** FOR THE DISCOVERY OF POTENTIAL DRUG CANDIDATES AND **DETERMINING** THE SPECIFICITY THEREOF

DETD The present invention also provides methods for identifying potential drug candidates (and lead compounds) and **determining** the specificities thereof. For example, knowing that a polypeptide containing a WW domain and a recognition unit, e.g., a binding. . . on the polypeptide-recognition unit interaction, e.g., either as an agonist or as an antagonist (inhibitor) of the interaction. With this **assay**, then, one can screen a collection of candidate "drugs" for the one exhibiting the most desired characteristic, e.g., the most. . .

DETD In one embodiment, the effect of the drug candidate upon multiple, different interacting polypeptide-recognition unit pairs is **determined** in which at least some of said polypeptides have a WW domain that differs in sequence but is capable of. . .

DETD In another embodiment, the drug candidate is an inhibitor of the polypeptide-recognition unit interaction that is identified by **detecting** a decrease in the binding of polypeptide to recognition unit in the presence of such inhibitor.

DETD (iii) **determining** the amino acid sequence of the polypeptides

identified in step (ii); and
DETD (ii) **determining** a consensus sequence for the peptides obtained in step (i);
DETD (v) **determining** the amino acid sequence of the polypeptides identified in step (iv); and
DETD In a preferred embodiment, the effect of the drug candidate upon multiple, different interacting polypeptide-recognition unit pairs is **determined** in which preferably at least some (e.g., at least 2, 3, 4, 5, 7, or 10) of said polypeptides have. . . the methods of the present invention. In a specific embodiment, an inhibitor of the polypeptide-recognition unit interaction is identified by **detecting** a decrease in the binding of polypeptide to recognition unit in the presence of such inhibitor.
DETD . . . testing of man-made compounds. Typically, hundreds, or even thousands, of compounds are randomly screened by the use of in vitro **assays** such as those that monitor the compound's effect on some enzymatic activity, its ability to bind to a reference substance. . .
DETD There is a continual need for new compounds to be tested in the in vitro **assays** that make up the first testing step described above. There is also a continual need for new **assays** by which the pharmacological activities of these compounds may be tested. It is an object of the present invention to provide such new **assays** to **determine** whether a candidate compound is capable of affecting the binding between a polypeptide containing a WW domain and a recognition. . . invention to provide polypeptides, particularly novel ones, containing WW domains and their corresponding recognition units for use in the above-described **assays**. The use of these polypeptides greatly expands the number of **assays** that may be used to screen potential drug candidates for useful pharmacological activities (as well as to identify potential drug. . .
DETD (iii) **determining** the amino acid sequence of the polypeptides identified in step (ii); and
DETD (ii) **determining** a consensus sequence for the peptides obtained in step (i);
DETD (v) **determining** the amino acid sequence of the novel polypeptides identified in step (iv); and
DETD . . . recognize that it will not always be necessary to utilize the entire novel polypeptide containing the WW domain in the **assays** described herein. Often, a portion of the polypeptide that contains the WW domain will be sufficient, e.g., a glutathione S-transferase. . .
DETD A typical **assay** of the present invention consists of at least the following components: (1) a molecule (e.g., protein or polypeptide) comprising a. . . of having the capacity to affect the binding between the protein containing the WW domain and the recognition unit. The **assay** components may further comprise (4) a means of **detecting** the binding of the protein comprising the WW domain and the recognition unit. Such means can be e.g., a **detectable** label affixed to the protein comprising the WW domain, the recognition unit, or the candidate compound. In a specific embodiment,. . .
DETD . . . the WW domain and the recognition unit under conditions conducive to binding in the presence of a candidate compound and **measuring** the amount of binding between the molecule and the recognition unit;
DETD (b) comparing the amount of binding in step (a) with the amount of binding known or **determined** to occur between the molecule and the recognition unit in the absence of the candidate compound, where a difference in the amount of binding between step (a) and the amount of binding known or **determined** to occur between the molecule and the recognition unit in the absence of the candidate compound indicates that the candidate. . .
DETD . . . the WW domain and the recognition unit under conditions conducive to binding in the presence of a candidate compound and **measuring** the amount of binding between the molecule and the recognition unit in which the WW domain has an amino acid. . .
DETD (b) comparing the amount of binding in step (a) with the amount of binding known or **determined** to occur between the molecule and the recognition unit in the absence of the candidate compound, where a difference in the amount of binding between step (a) and the amount of binding known or **determined** to occur between the molecule and the recognition unit in the absence of the candidate compound indicates

that the candidate. . .

DETD In one embodiment, the **assay** comprises allowing the polypeptide containing a WW domain to contact a recognition unit that selectively binds to the WW domain. . . the polypeptide containing a WW domain will occur unless that binding is disrupted or prevented by the candidate compound. By **detecting** the amount of binding of the recognition unit to the polypeptide containing a WW domain in the presence of the. . . recognition unit to the polypeptide containing a WW domain in the absence of the candidate compound, it is possible to **determine** whether the candidate compound affects the binding and thus is a useful lead compound for the modulation of the activity. . .

DETD One version of an **assay** suitable for use in the present invention comprises binding the polypeptide containing a WW domain to a solid support such. . . labeled recognition unit will bind to the polypeptide containing a WW domain in the well. This binding can then be **detected**. If the candidate compound interferes with the binding of the polypeptide containing a WE domain and the labeled recognition unit,. . . WW domain and the labeled recognition unit. Alternatively, the recognition unit can be affixed to a solid substrate during the **assay**.

DETD . . . WW domains. For each candidate drug compound, a table such as Table 1 is generated from the results of binding **assays**. An X placed at the intersection of a particular numbered row and lettered column represents a positive **assay** for binding, i.e., the candidate drug compound affected the binding of the recognition unit of that particular row to the. . .

DETD Such data as that illustrated above is used to **determine** whether novel polypeptides or other molecules display or are at risk of displaying desirable or undesirable physiological or pharmacological activities.. . .

DETD Accordingly, the present invention provides a method of utilizing the polypeptides comprising WW domains of the present invention in an **assay** to **determine** the participation of those polypeptides in pharmacological activities.

DETD (a) contacting a drug candidate with a molecule comprising a WW domain under conditions conducive to binding, and **detecting** or **measuring** any specific binding that occurs; and

DETD The present invention also provides a method of **determining** the potential pharmacological activities of a molecule comprising:

DETD (b) **detecting** or **measuring** any specific binding that occurs; and

DETD . . . the clonal bacteriophage from the isolated plaques may be tested against each of the biotinylated peptides individually, in order to **determine** to which of the several peptides that were used as recognition units in the primary screen the phage are actually. . .

DETD . . . amino acid sequences of polypeptides comprising WW domains, preferably human polypeptides, and fragments and derivatives thereof which comprise an antigenic **determinant** (i.e., can be recognized by an **antibody**) or which are functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" material as used herein. . . refers to that material displaying one or more functional activities, e.g., a biological activity, antigenicity (capable of binding to an **antibody**) immunogenicity, or comprising a WW domain that is capable of specific binding to a recognition unit. In specific embodiments, the. . .

DETD . . . be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be **detected** by **assays** based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which. . . ("adhesiveness") or antigenic properties as known for the particular polypeptide comprising a WW domain from the first species. If an **antibody** to that particular polypeptide is available, the corresponding polypeptide from another species may be identified by binding of labeled **antibody** to the putative polypeptide synthesizing clones in an ELISA (enzyme-linked immunosorbent **assay**)-type procedure.

DETD . . . DNA fragments may represent available, purified DNA of genes encoding polypeptides comprising a WW domain of a first species. Immunoprecipitation **analysis** or functional **assays** (e.g., ability to bind to a recognition unit) of the in vitro

translation products of the isolated mRNAs identifies the. . . contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized **antibodies** specifically directed against polypeptides comprising a WW domain. A radiolabelled cDNA of a gene encoding a polypeptide comprising a WW. . .

DETD . . . 38, 647-658; Adames et al., 1985, Nature 318, 533-538; Alexander et al., 1987, Mol. Cell. Biol. 7, 1436-1444), mouse mammary **tumor** virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45, 485-495),. . .

DETD . . . of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be **detected** by nucleic acid hybridization using probes comprising sequences that are homologous to the inserted gene. In the second approach, the. . . identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by **assaying** the foreign gene product expressed by the recombinant. Such **assays** can be based, for example, on the physical or functional properties of the gene product in in vitro **assay** systems, e.g., ability to bind to recognition units.

DETD . . . recombinant which expresses the gene sequence encoding a polypeptide comprising a WW domain is identified, the gene product may be **analyzed**. This can be achieved by **assays** based on the physical or functional properties of the product, including radioactive labelling of the product followed by **analysis** by gel electrophoresis.

DETD . . . or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable **assay**, including, but not limited to, binding to a recognition unit.

DETD . . . during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an **antibody** molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but. . .

DETD 5.10 **ANTIBODIES** TO POLYPEPTIDES COMPRISING A WW DOMAIN

DETD According to one embodiment, the invention provides **antibodies** and fragments containing the binding domain thereof, directed against polypeptides comprising a WW domain. Accordingly, polypeptides comprising a WW domain, fragments, analogs, or derivatives thereof, in particular, may be used as immunogens to generate **antibodies** against such polypeptides, fragments, analogs, or derivatives. Such **antibodies** can be polyclonal, **monoclonal**, chimeric, single chain, Fab fragments, or from an Fab expression library. In a specific embodiment, **antibodies** specific to the WW domain of a polypeptide comprising a WW domain may be prepared.

DETD Various procedures known in the art may be used for the production of polyclonal **antibodies**. In a particular embodiment, rabbit polyclonal **antibodies** to an epitope of a polypeptide comprising a WW domain, or a subsequence thereof, can be obtained. For the production of **antibody**, various host animals can be immunized by injection with the native polypeptide comprising a WW domain, or a synthetic version,. . .

DETD For preparation of **monoclonal antibodies**, any technique which provides for the production of **antibody** molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and. . . the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4, 72), and the EBV-hybridoma technique to produce human **monoclonal antibodies** (Cole et al., 1985, in **Monoclonal Antibodies** and **Cancer** Therapy, Alan R. Liss, Inc., pp. 77-96) may be used.

DETD **Antibody** fragments which contain the idiotype (binding domain) of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragment which can be produced by pepsin digestion of the **antibody** molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragment, and the Fab fragments

which can be generated by treating the **antibody** molecule with papain and a reducing agent.

DETD In the production of **antibodies**, screening for the desired **antibody** can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent **assay**).

DETD A study was initiated to **determine** whether peptide recognition units could recognize WW domains that are the same as or similar to their target WW domain. . .

DETD . . . biotinylated and complexed with streptavidin-alkaline phosphatase as described above except for the WBP-1 peptide which was complexed with streptavidin-horseradish peroxidase. **Detection** of the bound peptides was as described above except for WBP-1, which was **detected** with the IBI enzygraphic.TM. Web (Kodak, New Haven, Conn.) as described by the manufacturer. See Section 6.5. Alternatively, the TSA. . .

DETD In addition to the WW domains, primary sequence **analysis** of the novel clones revealed several other interesting structural features. Clones WWP2 and WWP1, respectively, contain a complete and partial. . .

DETD . . . by centrifugation at 500.times. g for 5 minutes. The amount of fusion protein recovered was estimated by the Bradford protein **assay**, and its purity was evaluated by 10% SDS-polyacrylamide gel electrophoresis and Coomassie Blue staining.

DETD . . . vector displays a peptide sequence at the N-terminus of the mature pIII protein that encodes the epitope for the mouse **monoclonal antibody** 7E11 (see FIG. 12); it includes the stop codon TAG in the coding region, which is suppressed in E. coli. . .

DETD . . . dystrophin WW domain-binding phage were sequenced via standard DNA sequencing techniques and the corresponding amino acid sequences of the inserts **determined**. Six of these peptides corresponding to the **determined** sequences were synthesized and biotinylated. The sequences of these peptides are shown below.

DETD To **determine** the ligand preferences of the novel WW domain-containing clones described in Sections 6.1 and 6.1.1, as well as addressing the. . .

DETD . . . bound to the protein expressed by clone WWP3, suggesting that the single WW domain in this clone may recognize additional **determinants** outside of the core PPPPY (SEQ ID NO:3) motif. In addition, the WBP-2B peptide containing an N-terminal tyrosine residue had. . .

DETD 6.5. BIOTINYLATED PEPTIDE **DETECTION** USING TYRAMIDE AMPLIFICATION SYSTEM

DETD The following protocol is an alternative to the methods described herein that utilize alkaline phosphatase to **detect** the binding of recognition units and WW domains. It permits the use of recognition units that are phosphopeptides.

DETD . . . .mu.l 1 mg/ml stock per 20 ml SuperBlock). Exposure time and concentration of SA-AP to filters may have to be **determined** empirically. Use about 10 ml per filter.

=> d bib abs 7

L53 ANSWER 7 OF 14 MEDLINE
AN 2000285339 MEDLINE
DN 20285339 PubMed ID: 10824099
TI Perlecan domain V of Drosophila melanogaster. Sequence, recombinant **analysis** and tissue expression.
AU Friedrich M V; Schneider M; Timpl R; Baumgartner S
CS Max-Planck-Institut fur Biochemie, Germany.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (2000 Jun) 267 (11) 3149-59.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200007
ED Entered STN: 20000728
Last Updated on STN: 20000728
Entered Medline: 20000720
AB The C-terminal domain V of the basement membrane proteoglycan perlecan was previously shown to play a major role in extracellular matrix and cell interactions. A homologous sequence of 708 amino-acid residues from Drosophila has now been shown to be 33% identical to mouse perlecan domain V. It consists of three **laminin** G-type (LG) and epidermal growth factor-like (EG) modules but lacks the EG3 module and a link region found in mammalian perlecans. Recombinant production of Drosophila perlecan domain V in mammalian cells yielded a 100-kDa protein which was folded into a linear array of three globular LG domains. Unlike the mouse counterpart, domain V from Drosophila was not modified by glycosaminoglycans and endogenous proteolysis, due to the absence of the link region. It showed moderate affinities for heparin and sulfatides but did not bind to chick alpha-**dystroglycan** or to various mammalian basement membrane proteins. A single RGD sequence in LG3 of Drosophila domain V was also incapable of mediating cell adhesion. Production of a proteoglycan form of perlecan (approximately 450 kDa) in one Drosophila cell line could be demonstrated by immunoblotting with **antibodies** against Drosophila domain V. A strong expression was also found by in situ hybridization and immunohistology at various stages of embryonic development and expression was localized to several basement membrane zones. This indicates, as for mammalian species, a distinct role of perlecan during Drosophila development.

=> d bib abs 8

L53 ANSWER 8 OF 14 MEDLINE
AN 2001061077 MEDLINE
DN 20532526 PubMed ID: 11078877
TI Anomalous **dystroglycan** in **carcinoma** cell lines.
AU Losasso C; Di Tommaso F; Sgambato A; Ardito R; Cittadini A; Giardina B; Petrucci T C; Brancaccio A
CS Centro Chimica dei Recettori (CNR), Istituto di Chimica e Chimica Clinica, Universita Cattolica del Sacro Cuore, Rome, Italy.
SO FEBS LETTERS, (2000 Nov 10) 484 (3) 194-8.
Journal code: EUH. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200012
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001222
AB **Dystroglycan** is a receptor responsible for crucial interactions between extracellular matrix and cytoplasmic space. We provide the first evidence that **dystroglycan** is truncated. In HC11 normal murine and the 184B5 non-**tumorigenic** mammary human cell lines, the expected beta-**dystroglycan** 43 kDa band was found but human breast T47D, BT549, MCF7, colon HT29, HCT116, SW620, prostate DU145 and cervical HeLa **cancer** cells expressed an anomalous approximately 31 kDa beta-**dystroglycan** band. alpha-**Dystroglycan** was udetectable in most of the cell lines in which beta-**dystroglycan** was found as a approximately 31 kDa species. An anomalous approximately 31 kDa beta-**dystroglycan** band was also observed in N-methyl-N-nitrosurea-induced primary rat mammary tumours. Reverse transcriptase polymerase chain reaction experiments confirmed the absence of alternative splicing events and/or expression of eventual **dystroglycan** isoforms. Using protein extraction procedures at low- and high-ionic strength, we demonstrated that both the 43 kDa and approximately 31 kDa beta-**dystroglycan** bands harbour their transmembrane segment.

=> d bib abs 9

L53 ANSWER 9 OF 14 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 1999:777043 SCISEARCH
GA The Genuine Article (R) Number: 243VH
TI Characterization of the Shank family of synaptic proteins - Multiple genes, alternative splicing, and differential expression in brain and development
AU Lim S; Naisbitt S; Yoon J; Hwang J I; Suh P G; Sheng M; Kim E (Reprint)
CS PUSAN NATL UNIV, DEPT PHARMACOL, PUSAN 609735, SOUTH KOREA (Reprint); PUSAN NATL UNIV, DEPT PHARMACOL, PUSAN 609735, SOUTH KOREA; MASSACHUSETTS GEN HOSP, HOWARD HUGHES MED INST, BOSTON, MA 02114; HARVARD UNIV, SCH MED, BOSTON, MA 02114; POHANG UNIV SCI & TECHNOL, DEPT LIFE SCI, POHANG 790784, SOUTH KOREA
CYA SOUTH KOREA; USA
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (8 OCT 1999) Vol. 274, No. 41, pp. 29510-29518.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 51
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Shank1, Shank2, and Shank3 constitute a family of proteins that may function as molecular scaffolds in the postsynaptic density (PSD). Shank directly interacts with GKAP and Homer, thus potentially bridging the N-methyl-D-aspartate receptor-PSD-95-GKAP complex and the mGluR-Homer complex in synapses (Naisbitt, S., Kim, E., Tu, J. C., Xiao, B., Sala, S., Valtschanoff, J., Weinberg, R J., morley, P. F., and Sheng, M. (1999) Neuron 23, 569-582; Tu, J. C., Xiao, B., Naisbitt, S., Yuan, J. P., Petralia, R. S., Brakeman, P., Dean, A., Aakalu, V. K., Lanahan, A. A., Sheng, M., and Worley, P. F. (1999) Neuron 23, 583-592). Shank contains multiple domains for protein-protein interaction including ankyrin repeats, an SH3 domain, a PSD-95/DIg/ZO-1 domain, a sterile a motif domain, and a proline-rich region. By characterizing Shank cDNA clones and RT-PCR products, we found that there are four sites far alternative splicing in Shank1 and another four sites in Shank2, some of which result in deletion of specific domains of the Shank protein. In addition, the expression of the splice variants is differentially regulated in different regions of rat brain during development. Immunoblot **analysis** of Shank proteins in rat brain using five different Shank **antibodies** reveals marked heterogeneity in size (120-240 kDa) and differential. spatiotemporal expression. Shank1 immunoreactivity is concentrated at excitatory synaptic sites in adult brain, and the punctate staining of Shank1 is seen in developing rat brains as early as postnatal day 7. These results suggest that alternative splicing in the Shank family may be a mechanism that regulates the molecular structure of Shank and the spectrum of Shank-interacting proteins in the PSDs of adult and developing brain.

=> d bib abs 10

L53 ANSWER 10 OF 14 USPATFULL
AN 1998:119000 USPATFULL
TI Tyrosine kinase receptors and ligands
IN Valenzuela, David M., Franklin Square, NY, United States
Glass, David J., White Plains, NY, United States
Bowen, David C., Yonkers, NY, United States
Yancopoulos, George D., Yorktown Heights, NY, United States
PA Regeneron Pharmaceuticals, Inc., Tarrytown, NY, United States (U.S. corporation)
PI US 5814478 19980929
AI US 1996-644271 19960510 (8)
PRAI US 1995-8657 19951215 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Walsh, Stephen; Assistant Examiner: Sorensen, Kenneth A.
LREP Cobert, Robert J.
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 37 Drawing Figure(s); 23 Drawing Page(s)
LN.CNT 3104
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides for a gene, designated as musk, that encodes a novel tyrosine kinase receptor expressed in high levels in denervated muscle. The invention also provides for an isolated and purified polypeptide which activates MuSK receptor. The invention further provides for a polypeptide which is functionally equivalent to the MuSK activating polypeptide. The invention also provides **assay** systems that may be used to **detect** and/or **measure** ligands that bind the musk gene product. The present invention also provides for **diagnostic** and therapeutic methods based on molecules that activate MuSK.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic 10

L53 ANSWER 10 OF 14 USPATFULL

AB . . . The invention further provides for a polypeptide which is functionally equivalent to the MuSK activating polypeptide. The invention also provides **assay** systems that may be used to **detect** and/or **measure** ligands that bind the musk gene product. The present invention also provides for **diagnostic** and therapeutic methods based on molecules that activate MuSK.

SUMM . . . which transmits a biological signal to intracellular target proteins. The particular array of sequence motifs of this cytoplasmic, catalytic domain **determines** its access to potential kinase substrates (Mohammadi, et al., 1990, Mol. Cell. Biol., 11: 5068-5078; Fantl, et al., 1992, Cell,. . .

SUMM . . . brain tissue, although significant levels of trkB mRNAs were also observed in lung, muscle, and ovaries. Further, trkB transcripts were **detected** in mid and late gestation embryos. In situ hybridization **analysis** of 14 and 18 day old mouse embryos indicated that trkB transcripts were localized in the central and peripheral nervous. . .

SUMM . . . in proliferation of the fibroblast (Glass, et al., 1991, Cell 66:405-413). Thus, it appears that the extracellular domain provides the **determining** factor as to the ligand specificity, and once signal transduction is initiated the cellular environment will **determine** the phenotypic outcome of that signal transduction.

SUMM . . . Oncogene 8: 1631-1637). Isolation of such so called "orphan" tyrosine kinase receptors, for which no ligand is known, and subsequent **determination** of the tissues in which such receptors are expressed, provides insight into the regulation of the growth, proliferation and regeneration. . . isolation of such receptors, ligands and activating molecules enhances our understanding of developmental processes and may improve our ability to **diagnose** or treat abnormal conditions.

SUMM . . . lamina is distinguished from the adjacent extracellular matrix by the accumulation of a number of proteins, such as acetylcholinesterase and s-**laminin**. The synaptic basal lamina also serves as a reservoir for signaling molecules exchanged between nerve and muscle.

SUMM . . . 8: 691-699; Ferns, M., et al., 1993, Neuron 11: 491-502; Hoch, W., et al., 1993, Neuron 11: 479-490), and that **antibodies** to agrin block nerve-induced clustering of AChRs on cultured myotubes (Reist, N. E., et al., 1992, Neuron 8: 865-868).

SUMM Intriguing recent findings have revealed that agrin can directly bind to .alpha.-**dystroglycan**, an extrinsic peripheral membrane protein that is attached to the cell surface by covalent linkage to .beta.-**dystroglycan**, which in turn couples to the intracellular cytoskeletal scaffold via an associated protein complex (Bowe, M. A., et al., 1994,. . .

SUMM Extrasynaptically, the **dystroglycan** complex binds **laminin** on its extracellular face, and couples to the actin scaffold via a spectrin-like molecule known as dystrophin. At the synapse however, agrin (via its own **laminin**-like domains) may be able to substitute for **laminin**, whereas utrophin (a dystrophin related protein) replaces dystrophin as the link to actin (reviewed in (Bowe, M. A. and Fallon, J. R., 1995, Ann. Rev. Neurosci. 18: 443-462)). The **dystroglycan** complex co-clusters with AChRs in response to agrin in vitro, and components of this complex are concentrated at the endplate. . .

SUMM . . . a 43 kD cytoplasmic protein, known as rapsyn, anchors AChRs to a sub-synaptic cytoskeleton complex, probably via interactions with the **dystroglycan** complex (Cartaud, J. and Changeux, J. P., 1993, Eur. J. Neurosci. 5: 191-202; Apel, E. D., et al., 1995, Neuron. . . 15: 115-126). Gene disruption studies reveal that rapsyn is absolutely necessary for clustering of AChRs, as well as of the **dystroglycan** complex. However, other aspects of NMJ formation, involving presynaptic differentiation and synapsespecific transcription, are seen in mice lacking rapsyn (Gautam,. . .

SUMM Despite the findings that agrin can bind directly to .alpha.-**dystroglycan**, and that AChRs and the **dystroglycan**

complex are linked and co-cluster in response to agrin, the role of **dystroglycan** as an agrin receptor remains unclear (Sealock, R. and Froehner, S. C., 1994, Cell 77: 617-619; Ferns, M., et al.,. . . fragment of chick agrin is sufficient to induce AChR aggregation (Gesemann, M., et al., 1995, J. Cell. Biol. 128: 625-636). **Dystroglycan** could be directly involved in activating signaling pathways that appear to be required for clustering, such as those involving tyrosine. . .

SUMM Alternatively, **dystroglycan** could be involved in couplings of agrin not only to AChRs but to a novel signaling receptor. It also remains possible that **dystroglycan** does not play an active or required role in initiating clustering, and is merely among an assortment of post-synaptic molecules. . . undergo clustering. Recent evidence indicates that the agrin fragment that is active in inducing AChR aggregation does not bind to .alpha.-**dystroglycan** and a structural role in aggregation, rather than a signal transfer role, has been proposed for the binding of agrin to .alpha.-**dystroglycan** (Gesemann, M., et al., 1996, Neuron 16: 755-767).

SUMM . . . cognate ligands and activating molecules. For example, the MuSK receptor activating molecule described herein may be used in a competition **assay** to identify agents capable of acting as receptor agonists or antagonists by competing the agents with MuSK activating molecule for. . . Specifically, the active portion of human agrin described herein may be used as the MuSK activating molecule in a competition **assay** to screen for agents capable of acting as receptor agonists or antagonists.

SUMM . . . of hybridizing with a sequence included within the nucleotide sequence encoding human MuSK or its activating molecule, useful for the **detection** of MuSK expressing tissue or MuSK activating molecule-expressing tissue in humans and animals. The invention further provides for **antibodies** capable of specifically binding MuSK or MuSK activating molecule. The **antibodies** may be polyclonal or **monoclonal**.

SUMM The present invention also has **diagnostic** and therapeutic utilities. In particular embodiments of the invention, methods of **detecting** aberrancies in the function or expression of the receptor described herein may be used in the **diagnosis** of muscular or other disorders. In other embodiments, manipulation of the receptor, agonists which bind this receptor, or receptor activating. .

SUMM The present invention also includes an **antibody** capable of specifically binding human agrin. More specifically, the invention includes an **antibody** capable of specifically binding the active portion of human agrin. The **antibody** may be **monoclonal** or polyclonal. The invention further provides a method of **detecting** the presence of human agrin in a sample comprising:

SUMM a) reacting the sample with an **antibody** capable of specifically binding human agrin under conditions whereby the **antibody** binds to human agrin present in the sample; and

SUMM b) **detecting** the bound **antibody**, thereby **detecting** the presence of human agrin in the sample.

SUMM The **antibody** used may be **monoclonal** or polyclonal. The sample may be biological tissue or body fluid. The biological tissue may be brain, muscle, or spinal. . .

DRWD . . . The novel EcoRI (R) and NcoI (N) fragments generated following successful targeting are labeled. The 5' EcoRI/HpaI probe used to **detect** the endogenous and mutant EcoRI fragments was derived from genomic DNA not included in the targeting construct. B, BamHI; Hp,. . .

DRWD . . . Knockout Mice--Southern blot of tail DNA from wild-type, heterozygous and homozygous F2 progeny showing the endogenous and mutant EcoRI fragments **detected** by the 5' RI/HpaI probe, as well as the endogenous NcoI fragments **detected** by the kinase region probe, which are absent in the homozygous mutant.

DRWD FIGS. 7A-7D Post-mortem histological **analysis** of lung demonstrating that the alveoli air sacs in the MuSK.sup.-/- newborn are not expanded (FIG. 7A) as they are. . . the lung of the control littermate (FIG. 7B), indicating that mutant pups do not take a single breath. Post-mortem histological **analysis** of hindlimb

musculature reveals that MuSK.sup.-/- mice (FIG. 7C) possess grossly normal muscle architecture similar to that of control mice. . .

DRWD . . . (FIG. 8B, each point represents the mean.+-.SEM of forty myotube segments). Total AChRs on the myotubes before agrin treatment was **determined** by binding with .sup.125 I-.alpha.-BGT (FIG. 8C, each bar represents the mean.+-.SEM CPM bound per .mu.g of total cell protein. . .

DRWD FIGS. 10A & 10B Agrin can not **detectably** bind to the isolated ectodomain of MuSK. Agrin was **assayed** for its binding to immobilized MuSK-Fc or to an immoblized agrin-specific **monoclonal antibody** (mAb), each coupled to a BIAcore sensorchip surface (FIG. 10A); bindings to the MuSK-Fc surface were also done in the presence 2 mM Ca.sup.++ or heparin (0.01 mg/ml), as indicated, while bindings to the **antibody** surface were also competed with excess soluble **monoclonal antibody** or MuSK-Fc (each at 25 .mu.g/ml), as indicated. Reciprocally, binding of soluble MuSK-Fc or **monoclonal antibody** to immobilized agrin was **assayed** by first binding conditioned media transfected with a plasmid control (Mock) or a plasmid encoding c-agrin4,8 (cAg.sub.4,8) to nitrocellulose, followed by **detection** using either the soluble MuSK-Fc or the agrin-specific **monoclonal antibody**, as indicated (FIG. 10B); TrkB-Fc **detection** of nitrocellulose-immobilized BDNF served as an additional control.

DRWD . . . differentiated cells (bottom panel). However, the chick MuSK can only be inducibly phosphorylated in response to agrin when it is **assayed** in differentiated myotubes (top panel). The chick MuSK displays the same specificity for activation by the various agrin isoforms (each. . .

DRWD . . . surface via only one of its components, the non-signaling a component; surface binding of the soluble b components can be **detected** using **antibodies** recognizing the Fc tag. FIG. 12C--Schematic representation of one of several possible models of the MuSK receptor complex for agrin,. . . or coupling to various effectors or substrates; these couplings may be mediated extracellularly (for example via agrin binding to the **dystroglycan** complex) or intracellularly (for example via interactions of SH2 domain-containing proteins to phosphorylated tyrosines on MuSK).

DRWD . . . accessory component. FIG. 13A--Formation of agrin/MuSK complexes on the surface of myotubes: undifferentiated (Undiff.) or myotube-differentiated (Diff) C2C12 cells were **assayed** for their ability to bind either MuSK-Fc or a control receptor-Fc fusion (TrkB-Fc), in the absence or presence of various. . . involve complexes analogous to those depicted in FIG. 12B. FIG. 13B--Direct binding of agrin to MuSK is demonstrated by cross-linking **analysis**. Radiolabelled agrin (a recombinant c-terminal fragment of human agrin, termed hAgrin.sub.4,8) at 1 nM was chemically cross-linked to the surface of myotubes. Following cross-linking, lysates were immunoprecipitated with a MuSK-specific **antibody** (lane 1). The cross-linking was also done in the presence of excess (150 nM) unlabelled agrin (lane 2), while the immunoprecipitation was also done in the presence of excess peptide (corresponding to that used to generate the **antibody**) to block the MuSK precipitation; positions of the agrin/MuSK complex, as well as of various forms of unbound monomeric and. . .

DETD The gene encoding rat MuSK has been cloned and the DNA sequence **determined** (FIGS. 1A-1D; SEQ ID NO: 2). The extracellular domain of the mature protein is believed to be encoded by the. . .

DETD The invention also provides for an **antibody** which specifically binds the above-described MuSK receptor. The **antibody** of the invention may be a polyclonal or **monoclonal antibody**

.

DETD The invention further provides a method of **detecting** the presence of MuSK ligand in a sample comprising:

DETD b) **detecting** the bound MuSK receptorbody, thereby **detecting** the presence of MuSK ligand in the sample.

DETD . . . fragments or derivatives thereof which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an **antibody** molecule or other cellular ligand, etc.

DETD . . . Substantial similarity at the protein level includes the ability of a subject protein to compete with MuSK for binding to **monoclonal antibodies** raised against MuSK epitopes.

DETD The MuSK protein described herein is useful in 1) screening strategies, 2) purification strategies and 3) **diagnostic** uses. With respect to screening strategies, expression cloning strategies based on cell survival and proliferation **assays** provide a method of screening for cognate ligands (Glass, et al. (1991) Cell 66:405-413). Since ligands that bind MuSK may. . .

DETD In other embodiments, the extracellular portion of RTKs that bind known ligands are replaced with the extracellular portion of MuSK. **Measurable** effects, such as changes in phenotype or induction of early response genes, normally associated with binding of the known ligand. . .

DETD . . . and eventually purify agents acting on that receptor. Once a particular receptor/ligand system is defined, a variety of additional specific **assay** systems can be utilized, for example, to search for additional agonists or antagonists of MuSK.

DETD . . . a MuSK binding ligand. Appropriate cell lines can be chosen to yield a response of the greatest utility for the **assay**, as well as discovery of agents that can act on tyrosine kinase receptors. "Agents" refers to any molecule(s), including but. . .

DETD . . . fibroblast cell line. Such a receptor which does not normally mediate proliferative responses may, following introduction into fibroblasts, nonetheless be **assayed** by a variety of well established methods used to quantitate effects of fibroblast growth factors (e.g. thymidine incorporation or other types of proliferation **assays**; see van Zoelen, 1990, "The Use of Biological **Assays** For **Detection** Of Polypeptide Growth Factors" in Progress in Factor Research, Vol. 2, pp. 131-152; Zhan and M. Goldfarb, 1986, Mol. Cell. Biol., Vol. 6, pp. 3541-3544). These **assays** have the added advantage that any preparation can be **assayed** both on the cell line having the introduced receptor as well as the parental cell line lacking the receptor. Only. . .

DETD The specific binding of test agent to the receptor may be **measured** in a number of ways. For example, the binding of test agent to cells may be **detected** or **measured**, by **detecting** or **measuring** (i) test agent bound to the surface of intact cells; (ii) test agent cross-linked to receptor protein in cell lysates;. . .

DETD Alternatively, the specific activity of test agent on the receptor may be **measured** by evaluating the secondary biological effects of that activity, including, but not limited to, the induction of neurite sprouting, immediate. . . but not in comparable cells that lack the receptor would be indicative of a specific test agent/receptor interaction. A similar **analysis** could be performed by **detecting** immediate early gene (e.g. fos and jun) induction in receptor-minus and receptor-plus cells, or by **detecting** phosphorylation of the receptor protein using standard phosphorylation **assays** known in the art.

DETD . . . comprising (i) exposing a cell that expresses a tyrosine kinase receptor as described herein to a test agent and (ii) **detecting** the activity of the test agent to the receptor, in which activity positively correlates with signal transducing activity. Activity may be **detected** by either **assaying** for direct binding or the secondary biological effects of binding, as discussed supra. Such a method may be particularly useful. . .

DETD The present invention also provides for **assay** systems that may be used according to the methods described supra. Such **assay** systems may comprise in vitro preparations of receptor, e.g. affixed to a solid support, or may preferably comprise cells that. . .

DETD . . . al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary **tumor** virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin. . .

DETD . . . of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be **detected** by DNA-DNA hybridization using probes comprising sequences that are homologous to an inserted gene. In the second approach, the recombinant.

. . identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by **assaying** the foreign gene product expressed by the recombinant vector. Such **assays** can be based, for example, on the physical or functional properties of the receptor-encoding gene product, for example, by binding of the receptor to neurotrophic factor or to an **antibody** which directly recognizes the receptor. Cells of the present invention may transiently or, preferably, constitutively and permanently express receptors or. . .

DETD . . . to the receptor, the binding secondarily induces transcription off the immediate early promoter. Such a cell may be used to **detect** receptor/ligand binding by **measuring** the transcriptional activity of the immediate early gene promoter, for example, by nuclear run-off **analysis**, Northern blot **analysis**, or by **measuring** levels of a gene controlled by the promoter. The immediate early promoter may be used to control the expression of fos or jun or any **detectable** gene product, including, but not limited to, any of the known reporter genes, such as a gene that confers hygromycin. . . and Efstratiadis, 1987, Proc. Natl. Acad. Sci. U.S.A. 84:8277-8281) chloramphenicol acetyltransferase (CAT), neomycin phosphotransferase (neo), beta-galactosidase beta-glucuronidase, beta-galactosidase, etc. of **detecting** or **measuring** neurotrophin activity.

DETD Furthermore, the cells used in the **assay** systems of the invention may or may not be cells of the nervous system. For example, in a specific, nonlimiting embodiment of the invention, growth-factor dependent fibroblasts may be used as the basis for a signal transducing **assay** system. A fibroblast cell line that is growth factor dependent in serum-free media (e.g. as described in Zham and Goldfarb, . .

DETD . . . biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an **antibody** molecule or other cellular ligand, etc.

DETD . . . cognate ligands and activating molecules. For example, the MuSK receptor activating molecule described herein may be used in a competition **assay** to identify agents capable of acting as receptor agonists or antagonists by competing the agents with MuSK activating molecule for. . . Specifically, the active portion of human agrin described herein may be used as the MuSK activating molecule in a competition **assay** to screen for agents capable of acting as receptor agonists or antagonists.

DETD . . . of hybridizing with a sequence included within the nucleotide sequence encoding human MuSK or its activating molecule, useful for the **detection** of MuSK expressing tissue or MuSK activating molecule-expressing tissue in humans and animals. The invention further provides for **antibodies** capable of specifically binding MuSK or MuSK activating molecule. The **antibodies** may be polyclonal or **monoclonal**.

DETD The present invention also has **diagnostic** and therapeutic utilities. In particular embodiments of the invention, methods of **detecting** aberrancies in the function or expression of the receptor described herein may be used in the **diagnosis** of muscular or other disorders. In other embodiments, manipulation of the receptor, agonists which bind this receptor, or receptor activating. .

DETD The present invention also includes an **antibody** capable of specifically binding human agrin. More specifically, the invention includes an **antibody** capable of specifically binding the active portion of human agrin. The **antibody** may be **monoclonal** or polyclonal. The invention further provides a method of **detecting** the presence of human agrin in a sample comprising:

DETD a) reacting the sample with an **antibody** capable of specifically binding human agrin under conditions whereby the **antibody** binds to human agrin present in the sample; and

DETD b) **detecting** the bound **antibody**, thereby **detecting** the presence of human agrin in the sample.

DETD The **antibody** used may be **monoclonal** or polyclonal. The sample may be biological tissue or body fluid. The biological tissue

may be brain, muscle, or spinal. . .

DETD . . . According to the present invention, probes capable of recognizing these receptors may be used to identify diseases or disorders by **measuring** altered levels of the receptor in cells and tissues. Such diseases or disorders may, in turn, be treatable using the. . . another embodiment, the muscle atrophy results from metabolic stress or nutritional insufficiency, including, but not limited to, the cachexia of **cancer** and other chronic illnesses, fasting or rhabdomyolysis, endocrine disorders such as, but not limited to, disorders of the thyroid gland. . .

DETD . . . humans, the disease known as idiopathic torsion dystonia (ITD) is associated with a gene that has been mapped, through linkage **analysis** to human chromosome 9q band 34. This disease is characterized by sustained, involuntary muscle contractions, frequently causing twisting and repetitive. . .

DETD . . . of such gene in situ. Alternatively, probes utilizing a unique segment of the musk gene may prove useful as a **diagnostic** for such disorders.

DETD The present invention provides for a method of **diagnosing** a neurological or other disorder in a patient comprising comparing the levels of expression of MuSK in a patient sample. . .

DETD One variety of probe which may be used is anti-MuSK **antibody** or fragments thereof containing the binding domain of the **antibody**.

DETD According to the invention, MuSK protein, or fragments or derivatives thereof, may be used as an immunogen to generate anti-MuSK **antibodies**. By providing for the production of relatively abundant amounts of MuSK protein using recombinant techniques for protein synthesis (based upon. . .

DETD To further improve the likelihood of producing an anti-MuSK immune response, the amino acid sequence of MuSK may be **analyzed** in order to identify portions of the molecule which may be associated with increased immunogenicity. For example, the amino acid sequence may be subjected to computer **analysis** to identify surface epitopes which present computer-generated plots of hydrophilicity, surface probability, flexibility, antigenic index, amphiphilic helix, amphiphilic sheet, and. . .

DETD For preparation of **monoclonal antibodies** directed toward MuSK, or its activating molecule, any technique which provides for the production of **antibody** molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and. . . technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human **monoclonal antibodies** (Cole et al., 1985, in "**Monoclonal Antibodies** and **Cancer** Therapy," Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

DETD The **monoclonal antibodies** for therapeutic use may be human **monoclonal antibodies** or chimeric human-mouse (or other species) **monoclonal antibodies**. Human **monoclonal antibodies** may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; Olsson et al., 1982, Meth. Enzymol. 92:3-16). Chimeric **antibody** molecules may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad.. . .

DETD Various procedures known in the art may be used for the production of polyclonal **antibodies** to epitopes of MuSK. For the production of **antibody**, various host animals can be immunized by injection with MuSK protein, or a fragment or derivative thereof, including but not. . .

DETD A molecular clone of an **antibody** to a MuSK epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular. . . Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) may be used to construct nucleotide sequences which encode a **monoclonal antibody** molecule, or antigen binding region thereof.

DETD **Antibody** molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid. . .

DETD The present invention provides for **antibody** molecules as well as fragments of such **antibody** molecules. **Antibody** fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab').sub.2 fragment which can be produced by pepsin digestion of the **antibody** molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragment, and the Fab fragments which can be generated by treating the **antibody** molecule with papain and a reducing agent.

DETD . . . to identify the expression of musk by aberrant tissues, such as malignancies. In additional embodiments, these methods may be used **diagnostically** to compare the expression of musk in cells, fluids, or tissue from a patient suffering from a disorder with comparable. . . .

DETD . . . and used for PCR using vector primers (T3 and T7) flanking the tyrosine kinase insert and these PCR fragments were **analyzed** by sequencing.

DETD . . . Ampicillin-resistant bacterial colonies from PCR transformation are inoculated into 96-well microtiter plates and individual colonies from these PCR clones are **analyzed** by sequencing of plasmid DNAs that are purified by standard plasmid miniprep procedures.

DETD A 680 nts fragment, containing the tyrosine kinase domain of MuSK, was radiolabeled and utilized in Northern **analysis** of various rat tissue specific RNAs. The rat tissue specific RNAs were fractionated by electrophoresis through a 1% agarose-formaldehyde gel. . . X-ray film at -70.degree. C. Ethidium bromide staining of the gel demonstrated that equivalent levels of total RNA were being **assayed** for the different samples.

DETD . . . construct was predicted to result in the generation of a novel 3.8 kb EcoRI fragment from the targeted allele as **detected** by a 5' probe, as well as loss of two NcoI fragments hybridizing to a kinase probe (FIG. 5). Southern. . .

DETD . . . gene disruption (designated MuSK-/- mice). Among the F2 litters derived from these crosses were newborn mice that died perinatally. Genotype **analysis** of tail DNA mice revealed that the dead pups were homozygous for the mutant MuSK allele (FIG. 6); significantly, not. . .

DETD To **determine** the phenotype of the MuSK-/- newborns immediately at birth, applicants were careful to observe the births of several litters derived. . . at birth and appeared not to breathe, although their hearts continued to beat for a short time after birth. To **determine** whether the MuSK-/- pups had ever taken a breath, applicants examined the lungs histologically. Lung alveoli are collapsed in utero,. . .

DETD . . . PBS, and permeabilized with 0.5% Triton X-100 in PBS (PBT) for 5 minutes. The muscles were then incubated with rabbit **antibodies** to synaptophysin (kindly provided by Dr. R. Jahn, Yale University Medical School), which were diluted 1/1000 in PBT with 2%. . . .

DETD . . . isolated myoblasts from newborn MuSK-/- mice or from control pups, attempted to differentiate them into myotubes in culture, and then **assayed** for their responsiveness to agrin.

DETD For agrin-mediated AChR clustering **assays** on primary myotubes, cultures on chamber slides were treated overnight with c-agrin4,8 at 0.01-100 nM; for evaluating MuSK-Fc as an. . . room temperature, washing, and then lysing the cells in 0.1N NaOH. The protein concentration in aliquots of each extract was **determined** using a BCA protein **assay** kit (Pierce),while the remainder of the extract was counted in a gamma counter.

DETD . . . in MuSK-/- myotubes even after increasing the concentration of c-agrin.sub.4,8 to as high as 100 nM (FIG. 8B). Lack of **detectable** clustering was not due to the absence of AChRs, since myotubes from MuSK-/- mice expressed similar numbers of AChR on. . .

DETD . . . advantage of the fact that RTKs become rapidly autophosphorylated on tyrosine upon challenge with their cognate ligand. Applicants decided to **assay** four of the known forms of soluble agrin--which exhibit differing AChR clustering activities (Ruegg, M. A. et al., 1992, Neuron. . .

DETD . . . to differentiate into myotubes in serum-poor media. After challenge, the cells were lysed, the extracts subjected to

immunoprecipitation with receptor-specific **antibodies**, and then immunoblotted with either receptor-specific or phosphotyrosine-specific **antibodies**, using methods previously described (Stitt, T., et al., 1995, Cell 80: 661-670). Polyclonal **antibodies** for MuSK were generated as follows: for rat MuSK, rabbits were immunized with a peptide corresponding to the carboxy-terminal 20 amino acids of the rat MuSK protein (Valenzuela, D., et al., 1995, Neuron 15: 573-584; the nomenclature for this **antibody** is: 41101K); for chick MuSK, rabbits were immunized with a peptide corresponding to the first 19 amino acids of the chick MuSK cytoplasmic domain (Peptide: TLPSELLLDRLHPNPMYQ (SEQ. ID. NO. 16); the nomenclature for this **antibody** is 52307K). The specificity of the **antibodies** was **determined** on Cos-cell expressed MuSK proteins, by both immune-precipitation and Western, comparing untransfected Cos cell lysates to lysates from rat and. . . 41101K immune precipitates and Westerns rodent MuSK, but does not recognize chicken MuSK. 52307 immune precipitates and Westerns chicken MuSK. **Antibodies** to ErbB3 were obtained from Santa Cruz Biotechnology, Inc.

DETD . . . mg/ml), each in the presence of the indicated mock or agrin-containing conditioned media (with 100 nM agrin). Agrin levels were **determined** by Western **analysis** of the conditioned media with a rat agrin **antibody** (131, from StressGen, Inc.), using a purified agrin control of known concentration. Following these incubations, the cells were washed four. . . and magnesium, and then incubated for an additional hour with radio-iodinated goat anti-human IgG (NEN/Dupont; 1 mCi/ml in PBS) to **detect** surface-bound receptor-Fc. After four additional washes, cells were solubilized in 0.1N NaOH, and bound radioactivity was **determined**. The **assay** is similar to that described elsewhere (Davis, S., et al., 1994, Science 266: 816-819).

DETD . . . similar to those seen for well-characterized RTK/ligand systems (e.g. Kaplan, D. R., et al., 1991, Nature 350: 158-160); induction was **detectable** by one minute, peaked within the first five minutes, and remained elevated for over an hour (FIG. 9D). The tyrosine. . . those noted for other ligands that act on RTKs (Ip, N. Y., et al., 1993, Neuron 10: 137-149), with phosphorylation **detectable** using 1 nM agrin (FIG. 9C).

DETD . . . agrin for MuSK as compared to other factors tested, and the precise correlation of agrin forms active in AChR clustering **assays** and in MuSK phosphorylation **assays**, together continue to support the notion that MuSK serves as the functional agrin receptor.

DETD The binding of agrin to immobilized MuSK-Fc as compared to a **monoclonal antibody** specific for agrin was evaluated by use of BIAcore biosensor technology (Pharmacia Biosensor), using approaches previously described (Stitt, T., et. . . 661-670). Heparin and CaCl2 were supplied by Sigma Chemical Co. (St. Louis, Mo.) and used without further purification. The agrin-specific **monoclonal antibody** (clone AGR131 generated to rat agrin) was purchased from StressGen Biotechnologies Corp. (Victoria, BC, Canada).

DETD . . . a first approach, applicants used MuSK-Fc together with BIAcore biosensor technology. The BIAcore technology allows for the direct and quantitative **measure** of binding of soluble ligands to receptors coupled onto a sensor chip. Recombinant MuSK-Fc was covalently coupled to a surface on the BIAcore sensor chip, and as a control, a **monoclonal antibody** specific for rat agrin was also coupled to a separate surface on the sensor chip; media containing c-agrin.sub.4,8 was then passed over the two surfaces. While robust binding of the agrin to the **antibody** surface was easily **detected**, no binding of the agrin to the MuSK surface could be seen (FIG. 10A). Furthermore, while binding to the **antibody** surface was specifically competable by excess soluble **antibody** added to the agrin-containing media, the binding was not competable by excess soluble MuSK-Fc (FIG. 10A). Since agrin activity requires. . .

DETD Next, applicants tried to demonstrate binding of MuSK and agrin by attempting to use MuSK-Fc to **detect** agrin immobilized onto nitrocellulose. In contrast to our control experiments, in which immobilized brain-derived neurotrophic factor (BDNF) was easily **detected** by an Fc fusion of its cognate receptor (TrkB-Fc), and

in which immobilized agrin was easily **detected** by the agrin-specific **monoclonal antibody**, immobilized agrin could not be **detected** by MuSK-Fc (FIG. 10B).

DETD . . . results described above, applicants considered the possibility that the agrin-MuSK interaction requires additional components. To further explore this possibility, applicants **determined** the cell-context dependency for agrin activation of MuSK, reasoning that if an accessory component was required, it might be specifically. . . cells normally responding to agrin. Thus applicants ectopically expressed full-length cDNAs encoding rat, human and chicken MuSK in fibroblasts, and **assayed** for whether these MuSK receptors could be inducibly phosphorylated by agrin. When expressed in fibroblasts, none of the three species. . . since the chicken MuSK could easily be distinguished from the endogenous mouse MuSK based on size and by using particular **antibodies**. When expressed in undifferentiated myoblasts, the chicken MuSK did not undergo phosphorylation in response to any isoforms of agrin (FIG.. . .

DETD . . . 80%; 125I-h-agrin 4,8-flg was separated from free 125I on a 1.times.3 cm Sephadex G-25 column prior to use in cross-linking **assays**. Specific activity was .about.4000 cpm/fmol (.about.2400 Ci/mmol). Biological activity of 125I-h-agrin 4,8-flg was monitored by tyrosine phosphorylation of MuSK in. . . 30 min, washed 3 times with 50 mM Tris/150 mM NaCl pH 7.5, lysed, and subjected to immunoprecipitation with MuSK-specific **antibodies**. For peptide competition, peptide antigen was included in the immunoprecipitation at a final concentration of 20 .mu.g/ml. The samples were. . .

DETD Immunoprecipitations using a MuSK-specific **antibody**, from lysates of myotubes chemically cross-linked to radiolabelled recombinant human agrin contained complexes corresponding in size to agrin/MuSK complexes (FIG.. . . or if a peptide was used to block MuSK precipitation (FIG. 13B). Additional radiolabelled species that immunoprecipitated with the MuSK **antibody** correspond to forms of agrin that are associated with, but not cross-linked to, MuSK, presumably due to the low efficiency of cross-linking (FIG. 13B); low levels of additional agrin complexes, perhaps involving MASC, could also be **detected** in these immunoprecipitations.

DETD . . . The crude soluble protein fraction containing human agrin 4-8, as well as human agrin 4-8 purified by Q-Sepharose chromatography was **determined** to be active in phosphorylation of MuSK receptor.

DETD . . . culture was centrifuged and the supernatant was dialyzed against PBS. The concentration of hAgrin was approximately 10 ug/ml and was **determined** to be active in phosphorylation of MuSK receptor.

DETD . . . C. in Gibco SF900 II serum-free medium. Uninfected cells were grown to a density of 1.times.10.sup.6 cells/mL. Cell density was **determined** by counting viable cells using a hemacytometer. The virus stock for FLAG-agrin was added to the bioreactor at a low. . .

DETD The virus titer was **determined** by plaque **assay** as described by O'Reilly, Miller and Luckow. The method is carried out in 60 mm tissue-culture dishes which are seeded. . .

DETD . . . concentrated 20-fold by diafiltration (DIAFLO ultrafiltration membranes, Amicon, Inc.). The quantity of active human agrin present in the media was **determined** and expressed as the amount (in resonance units, R.U.) of MuSK receptor specific binding activity **measured** by a BIAcore binding **assay**.

DETD . . . TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME/KEY: Other
(B) LOCATION: 1...18
(D) OTHER INFORMATION: Nomenclature for this **antibody** is 52307K.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
ThrLeuProSerGluLeuLeuLeuAspArgLeuHisProAsnProMet
151015
TyrGln

=> d bib abs 11

L53 ANSWER 11 OF 14 MEDLINE
AN 97298103 MEDLINE
DN 97298103 PubMed ID: 9153251
TI A role of **dystroglycan** in schwannoma cell adhesion to **laminin**.
AU Matsumura K; Chiba A; Yamada H; Fukuta-Ohi H; Fujita S; Endo T; Kobata A; Anderson L V; Kanazawa I; Campbell K P; Shimizu T
CS Department of Neurology and Neuroscience, Teikyo University School of Medicine, Tokyo 173, Japan.. k-matsu@med.teikyo-u.ac.jp
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 May 23) 272 (21) 13904-10. Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199707
ED Entered STN: 19970716
Last Updated on STN: 19970716
Entered Medline: 19970702
AB **Dystroglycan** is encoded by a single gene and cleaved into two proteins alpha- and beta-**dystroglycan** by posttranslational processing. Recently, alpha-**dystroglycan** was demonstrated to be an extracellular **laminin**-binding protein anchored to the cell membrane by a transmembrane protein beta-**dystroglycan** in striated muscle and Schwann cells. However, the biological functions of the **dystroglycan-laminin** interaction remain obscure, and in particular, it is still unclear if **dystroglycan** plays a role in cell adhesion. In the present study, we characterized the role of **dystroglycan** in the adhesion of schwannoma cells to **laminin**-1. Immunochemical **analysis** demonstrated that the **dystroglycan** complex, comprised of alpha- and beta-**dystroglycan**, was a major **laminin**-binding protein complex in the surface membrane of rat schwannoma cell line RT4. It also demonstrated the presence of alpha-**dystroglycan**, but not beta-**dystroglycan**, in the culture medium, suggesting secretion of alpha-**dystroglycan** by RT4 cells. RT4 cells cultured on dishes coated with **laminin**-1 became spindle in shape and adhered to the bottom surface tightly. **Monoclonal antibody** IIH6 against alpha-**dystroglycan** was shown previously to inhibit the binding of **laminin**-1 to alpha-**dystroglycan**. In the presence of IIH6, but not several other control **antibodies** in the culture medium, RT4 cells remained round in shape and did not adhere to the bottom surface. The adhesion of RT4 cells to dishes coated with fibronectin was not affected by IIH6. The known inhibitors of the interaction of alpha-**dystroglycan** with **laminin**-1, including EDTA, sulfatide, fucoidan, dextran sulfate, heparin, and sialic acid, also perturbed the adhesion of RT4 cells to **laminin**-1, whereas the reagents which do not inhibit the interaction, including dextran, chondroitin sulfate, dermatan sulfate, and GlcNAc, did not. Altogether, these results support a role for **dystroglycan** as a major cell adhesion molecule in the surface membrane of RT4 cells.

=> d bib abs 12

L53 ANSWER 12 OF 14 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 97:691857 SCISEARCH
GA The Genuine Article (R) Number: XV681
TI Sequence and functional relationships between androgen-binding protein sex hormone-binding globulin and its homologs protein S, Gas6, **laminin**, and agrin
AU Joseph D R (Reprint)
CS UNIV FLORIDA, DEV CTR BIOTECHNOL, APPL GENET LAB INC, 12085 RES DR, ALACHUA, FL 32615 (Reprint)
CYA USA
SO STEROIDS, (AUG-SEP 1997) Vol. 62, No. 8-9, pp. 578-588.
Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010.
ISSN: 0039-128X.
DT General Review; Journal
FS LIFE
LA English
REC Reference Count: 158
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Androgen-binding protein/sex hormone-binding globulin (ABP/SHBG) is an extracellular binding protein that regulates the bioavailability of sex steroids. ABP/SHBG is closely related to the globular (G) domain of vitamin K-dependent protein S family of proteins and more distantly related to the G domains of several extracellular matrix proteins. ABP/SHBG appears to have evolved from the fusion of two ancestral G domains. Expanding evidence suggests that ABP/SHBG has other functions that are mediated through membrane binding, including signal transduction; however, the types of binding proteins (receptors) have not been identified. Sequence comparisons of ABP/SHBG with G domains of its its homologs protein S, Gas6, **laminin**, and agrin have identified regions of ABP/SHBG that may bind receptors related to homolog receptors. These membrane receptors include beta-integrins, alpha-**dystroglycan**, and receptor tyrosine kinases. The G domains of **laminin** and related proteins have clearly evolved from a common ancestor to interact with specific receptors and binding proteins. It remains to be **determined** if ABP/SHBG followed this evolutionary pathway. (C) 1997 by Elsevier Science Inc.

=> d bib abs 13

L53 ANSWER 13 OF 14 MEDLINE
AN 96216490 MEDLINE
DN 96216490 PubMed ID: 8631999
TI Differential heparin inhibition of skeletal muscle alpha-**dystroglycan** binding to **laminins**.
AU Pall E A; Bolton K M; Ervasti J M
CS Department of Physiology, University of Wisconsin Medical School, Madison, Wisconsin 53706, USA.
NC AR42423 (NIAMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Feb 16) 271 (7) 3817-21.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199607
ED Entered STN: 19960715
Last Updated on STN: 19970203
Entered Medline: 19960702
AB The **laminin** binding properties of alpha-**dystroglycan** purified from rabbit skeletal muscle membranes were examined. In a solid phase microtiter **assay**, 125I-**laminin** (**laminin** -1) bound to purified alpha-**dystroglycan** in a specific and saturable manner with a half-maximal concentration of 8 nM. The binding of 125I- alpha-**dystroglycan** to native **laminin** and merosin (a mixture of **laminin**-2 and -4) was also compared using the solid phase **assay**. The absolute binding of 125I- alpha-**dystroglycan** to **laminin** (6955 +/- 250 cpm/well) was similar to that **measured** for merosin (7440 +/- 970 cpm/well). However, inclusion of 1 mg/ml heparin in the incubation medium inhibited 125I-alpha-**dystroglycan** binding to **laminin** by 84 +/- 4.3% but inhibited 125I-alpha-**dystroglycan** binding to merosin by only 17 +/- 5.2%. Similar results were obtained with heparan sulfate, while de-N-sulfated heparin, hyaluronic acid, and chondroitin sulfate had no differential effect. These results were confirmed by iodinated **laminin** and merosin overlay of electrophoretically separated and blotted dystrophin-glycoprotein complex. In contrast to the results obtained with skeletal muscle alpha-**dystroglycan**, both **laminin** and merosin binding to purified brain alpha-**dystroglycan** was significantly inhibited by heparin. Our data support the possibility that one or more heparan sulfate proteoglycans may specifically modulate the interaction of alpha-**dystroglycan** with different extracellular matrix proteins in skeletal muscle.

=> d bib abs 14

L53 ANSWER 14 OF 14 MEDLINE DUPLICATE 1
AN 95339960 MEDLINE
DN 95339960 PubMed ID: 7615068
TI Electron microscopic evidence for a mucin-like region in chick muscle alpha-**dystroglycan**.
AU Brancaccio A; Schulthess T; Gesemann M; Engel J
CS Department of Biophysical Chemistry, Biozentrum, University of Basel, Switzerland.
SO FEBS LETTERS, (1995 Jul 10) 368 (1) 139-42.
Journal code: EUH; 0155157. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199508
ED Entered STN: 19950905
Last Updated on STN: 19950905
Entered Medline: 19950824
AB alpha-**Dystroglycan** has been isolated from chicken cardiac muscle and its molecular weight was estimated to be approximately 135 kDa. The avian protein interacts with murine Engelbreth-Holm-Swarm (EHS) **tumor laminin** via interaction with the C-terminal LG4 and LG5 domains (fragment E3) of the **laminin** alpha-chain. This **laminin** binding is calcium-dependent and can be competed by heparin. Electron microscopy investigation on the shape of alpha-**dystroglycan** suggests that the core protein consists of two roughly globular domains connected by a segment which most likely corresponds to a mucin-like central region also predicted by sequence **analysis** on mammalian isoforms. This segment may act as a spacer in the dystrophin-associated glycoproteins complex exposing the N-terminal domain of alpha-**dystroglycan** to **laminin** in the extracellular space.

=> d bib abs 1

L55 ANSWER 1 OF 3 MEDLINE
AN 2001053440 MEDLINE
DN 20521694 PubMed ID: 11067874
TI Immunosuppression and resultant viral persistence by specific viral targeting of dendritic cells.
AU Sevilla N; Kunz S; Holz A; Lewicki H; Homann D; Yamada H; Campbell K P; de La Torre J C; Oldstone M B
CS Department of Neuropharmacology, Division of Virology, The Scripps Research Institute, La Jolla, California 92037, USA.
NC AG04342 (NIA)
AI09484 (NIAID)
AI45927 (NIAID)
+
SO JOURNAL OF EXPERIMENTAL MEDICINE, (2000 Nov 6) 192 (9) 1249-60.
Journal code: I2V. ISSN: 0022-1007.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200012
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001211
AB Among cells of the immune system, CD11c(+) and DEC-205(+) splenic dendritic cells primarily express the cellular receptor (alpha-**dystroglycan** [alpha-DG]) for lymphocytic choriomeningitis virus (LCMV). By selection, strains and variants of LCMV that bind alpha-DG with high affinity are associated with virus replication in the white pulp, show preferential replication in a majority of CD11c(+) and DEC-205(+) cells, cause immunosuppression, and establish a persistent infection. In contrast, viral strains and variants that bind with low affinity to alpha-DG are associated with viral replication in the red pulp, display minimal replication in CD11c(+) and DEC-205(+) cells, and generate a robust anti-LCMV cytotoxic T lymphocyte response that clears the virus infection. Differences in binding affinities can be mapped to a single amino acid change in the viral glycoprotein 1 ligand that binds to alpha-DG. These findings indicate that receptor-virus interaction on dendritic cells in vivo can be an essential step in the initiation of virus-induced immunosuppression and viral persistence.

=> d bib abs 2

L55 ANSWER 2 OF 3 MEDLINE DUPLICATE 1
AN 2001361079 MEDLINE
DN 21314141 PubMed ID: 11421342
TI A journey to the world of glycobiology.
AU Kobata A
CS Tokyo Metropolitan Institute of Gerontology, Japan.
SO GLYCOCONJUGATE JOURNAL, (2000 Jul-Sep) 17 (7-9) 443-64. Ref: 178
Journal code: BJJ; 8603310. ISSN: 0282-0080.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals
EM 200107
ED Entered STN: 20010730
Last Updated on STN: 20010730
Entered Medline: 20010726
AB Finding of the deletion phenomenon of certain oligosaccharides in human milk and its correlation to the blood types of the donors opened a way to elucidate the biochemical basis of blood types in man. This success led to the idea of establishing reliable techniques to elucidate the structures and functions of the N-linked sugar chains of glycoproteins. N-Linked sugar chains were first released quantitatively as oligosaccharides by enzymatic and chemical means, and labelled by reduction with NaB3H4. After fractionation, structures of the radioactive oligosaccharides were **determined** by a series of methods developed for the studies of milk oligosaccharides. By using such techniques, structural rules hidden in the N-linked sugar chains, and organ- and species-specific N-glycosylation of glycoproteins, which afforded a firm basis to the development of glycobiology, were elucidated. Finding of galactose deficiency in the N-linked sugar chains of serum IgG from patients with rheumatoid arthritis, and malignant alteration of N-glycosylation in various **tumors** opened a new research world called glycopathology. However, recent studies revealed that several structural exceptions occur in the sugar chains of particular glycoproteins. Finding of the occurrence of the Galbeta1-4Fucalpha1- group linked at the C-6 position of the proximal N-acetylglucosamine residue of the hybrid type sugar chains of octopus rhodopsin is one of such examples. This finding indicated that the fucosyl residue of the fucosylated trimannosyl core should no more be considered as a stop signal as has long been believed. Furthermore, recent studies on **dystroglycan** revealed that the sugar chains, which do not fall into the current classification of N and O-linked sugar chains, are essential for the expression of the functional role of this glycoprotein. It was found that expression of many glycoproteins is altered by aging. Among the alterations of the glycoprotein patterns found in the brain nervous system, the most prominent evidence was found in P0. This protein is produced in non-glycosylated form in the spinal cord of young mammals. However, it starts to be N-glycosylated in the spinal cord of aged animals. These evidences indicate that various unusual sugar chains occur as minor components in mammals, and play important roles in particular tissues.

=> d bib abs 3

L55 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 97:743978 SCISEARCH
GA The Genuine Article (R) Number: XZ016
TI Neuropilin-2, a novel member of the neuropilin family, is a high affinity receptor for the semaphorins Sema E and Sema IV but not Sema III
AU Chen H (Reprint); Chedotal A; He Z G; Goodman C S; TessierLavigne M
CS UNIV CALIF SAN FRANCISCO, HOWARD HUGHES MED INST, DEPT ANAT, DEPT BIOCHEM & BIOPHYS, SAN FRANCISCO, CA 94143 (Reprint); UNIV CALIF BERKELEY, HOWARD HUGHES MED INST, DEPT CELL & MOL BIOL, BERKELEY, CA 94720
CYA USA
SO NEURON, (SEP 1997) Vol. 19, No. 3, pp. 547-559.
Publisher: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138.
ISSN: 0896-6273.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Semaphorins are a targe family of secreted and transmembrane proteins, several of which are implicated in repulsive axon guidance. Neuropilin (neuropilin-1) was recently identified as a receptor for Collapsin-1/Semaphorin III/D (Sema III). We report the identification of a related protein, neuropilin-2, whose mRNA is expressed by developing neurons in a pattern largely, though not comptetely, nonoverlapping with that of neuropilin-1. Unlike neuropilin-1, which binds with high affinity to the th ree structurally related semaphorins Sema III, Sema E, and Sema IV, neuropilin-2 shows high affinity binding only to Sema E and Sema IV, not Sema III. These results identify neuropilins as a family of receptors (or components of receptors) for at least one semaphorin subfamily. They also suggest that the specificity of action of different members of this subfamily may be **determined** by the complement of neuropilins expressed by responsive cells.

=> d ind 3

L55 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 2001 ISI (R)
CC NEUROSCIENCES
STP KeyWords Plus (R): GROWTH CONE GUIDANCE; NERVOUS-SYSTEM; CELL-SURFACE; SPINAL-CORD; LUNG-**CANCER**; RECOGNITION MOLECULE; ALPHA-**DYSTROGLYCAN**; EXPRESSION; AGRIN; IDENTIFICATION
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

=> d bib abs 1-22

L70 ANSWER 1 OF 22 MEDLINE
AN 2000443748 MEDLINE
DN 20447456 PubMed ID: 10988240
TI Dystrophin associates with caveolae of rat cardiac myocytes: relationship to dystroglycan.
AU Doyle D D; Goings G; Upshaw-Earley J; Ambler S K; Mondul A; Palfrey H C; Page E
CS Department of Pharmacology and Physiology, University of Chicago, Chicago, IL, USA.
NC HL54302 (NHLBI)
SO CIRCULATION RESEARCH, (2000 Sep 15) 87 (6) 480-8.
Journal code: DAJ; 0047103. ISSN: 1524-4571.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200010
ED Entered STN: 20001012
Last Updated on STN: 20010521
Entered Medline: 20001003
AB The possibility of an interaction between the cytoskeletal protein dystrophin and **cell** surface caveolae in the mammalian myocardium was investigated by several techniques. Caveolin (cav)-3-enriched, detergent-insoluble membranes isolated from purified ventricular sarcolemma by density-gradient fractionation were found to contain dystrophin and dystroglycan. Further purification of cav-3-containing membranes by immunoprecipitation using anti-cav-3-coated magnetic beads yielded dystrophin but not always **dystroglycan**. Electron microscopic **analysis** of precipitated material revealed caveola-sized vesicular profiles that could be double-labeled with anti-dystrophin and anti-cav-3 **antibodies**. In contrast, immunoprecipitation of membranes with anti-dystrophin-coated beads yielded both cav-3 and **dystroglycan**. Electron microscopic **analysis** of this material showed heterogeneous membrane profiles, some of which could be decorated with anti-cav-3 **antibodies**. To confirm that dystrophin and cav-3 were closely associated in cardiac myocytes, we verified that dystrophin was also present in immunoprecipitated cav-3-containing membranes from detergent extracts, as well as in sonicated extracts of purified ventricular myocytes. Confocal immunofluorescence microscopy of ventricular and atrial cardiac myocytes showed that the cellular distributions of cav-3 and dystrophin partially overlapped. Immuno-electron micrographs of thin sections of rat atrial myocytes revealed a fraction of dystrophin molecules that are in apparently close apposition to caveolae. These results suggest that a subpopulation of dystrophin molecules interacts with cardiac myocyte caveolae in vivo and that some of the dystrophin is engaged in linking cav-3 with the dystroglycan complex.

L70 ANSWER 2 OF 22 MEDLINE
AN 1999196509 MEDLINE
DN 99196509 PubMed ID: 10098873
TI Association of the dystroglycan complex isolated from bovine brain synaptosomes with proteins involved in signal transduction.
AU Cavaldesi M; Macchia G; Barca S; Defilippi P; Tarone G; Petrucci T C
CS Laboratories of Cell Biology, Istituto Superiore di Sanita, Roma, Italy.
SO JOURNAL OF NEUROCHEMISTRY, (1999 Apr) 72 (4) 1648-55.
Journal code: JAV; 2985190R. ISSN: 0022-3042.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
ED Entered STN: 19990426
Last Updated on STN: 19990426
Entered Medline: 19990413
AB Dystroglycan is a transmembrane heterodimeric complex of alpha and beta subunits that links the extracellular matrix to the **cell** cytoskeleton. It was originally identified in skeletal muscle, where it

anchors dystrophin to the sarcolemma. Dystroglycan is also highly expressed in nonmuscle **tissues**, including brain. To investigate the molecular interactions of dystroglycan in the CNS, we fractionated a digitonin-soluble extract from bovine brain synaptosomes by laminin-affinity chromatography and characterized the protein components. The 120-kDa alpha-dystroglycan was the major 125I-laminin-labeled protein detected by overlay assay. This complex, in addition to beta-dystroglycan, was also found to contain Grb2 and focal adhesion kinase p125FAK (FAK). Anti-FAK **antibodies** co-immunoprecipitated Grb2 with FAK. However, no direct interaction between beta-**dystroglycan** and FAK was **detected** by co-precipitation assay. Grb2, an adaptor protein involved in signal transduction and cytoskeleton organization, has been shown to bind beta-dystroglycan. We isolated both FAK and Grb2 from synaptosomal extracts by chromatography on immobilized recombinant beta-dystroglycan. In the CNS, FAK phosphorylation has been linked to membrane depolarization and neurotransmitter receptor activation. At the synapses, the adaptor protein Grb2 may mediate FAK-beta-dystroglycan interaction, and it may play a role in transferring information between the dystroglycan complex and other signaling pathways.

L70 ANSWER 3 OF 22 MEDLINE
AN 1999182414 MEDLINE
DN 99182414 PubMed ID: 10080889
TI **Analysis** of heparin, alpha-**dystroglycan** and sulfatide binding to the G domain of the laminin alpha1 chain by site-directed mutagenesis.
AU Andac Z; Sasaki T; Mann K; Brancaccio A; Deutzmann R; Timpl R
CS Max-Planck-Institut fur Biochemie, Martinsried, D-82152, Germany.
SO JOURNAL OF MOLECULAR BIOLOGY, (1999 Mar 26) 287 (2) 253-64.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
ED Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990428
AB The 395-residue proteolytic **fragment** E3, which comprises the two most C-terminal LG modules of the mouse laminin alpha1 chain, was previously shown to contain major binding sites for heparin, alpha-dystroglycan and sulfatides. The same **fragment** (alpha1LG4-5) and its individual alpha1LG4 and alpha1LG5 modules have now been obtained by recombinant production in mammalian **cells**. These **fragments** were apparently folded into a native form, as shown by circular dichroism, electron microscopy and immunological assays. **Fragment** alpha1LG4-5 bound about five- to tenfold better to heparin, alpha-dystroglycan and sulfatides than E3. These binding activities could be exclusively localized to the alpha1LG4 module. Side-chain modifications and proteolysis demonstrated that Lys and Arg residues in the C-terminal region of alpha1LG4 are essential for heparin binding. This was confirmed by 14 single to triple point mutations, which identified three non-contiguous basic regions (positions 2766-2770, 2791-2793, 2819-2820) as contributing to both heparin and sulfatide binding. Two of these regions were also recognized by **monoclonal antibodies** which have previously been shown to inhibit heparin binding. The same three regions and a few additional basic residues also make major contributions to the binding of the cellular receptor alpha-dystroglycan, indicating a larger binding epitope. The data are also consistent with previous findings that heparin competes for alpha-dystroglycan binding.
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L70 ANSWER 4 OF 22 MEDLINE
AN 1998081499 MEDLINE
DN 98081499 PubMed ID: 9421146
TI Subcellular concentration of beta-dystroglycan in photoreceptors and glial **cells** of the chick retina.
AU Blank M; Koulen P; Kroger S
CS Department of Neuroanatomy, Max-Planck-Institute for Brain Research,

Frankfurt, Germany.
SO JOURNAL OF COMPARATIVE NEUROLOGY, (1997 Dec 29) 389 (4) 668-78.
Journal code: HUV; 0406041. ISSN: 0021-9967.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199802
ED Entered STN: 19980217
Last Updated on STN: 19980217
Entered Medline: 19980204
AB Mutations in the dystrophin-glycoprotein complex cause muscle degeneration and dysfunctions in the central nervous system, including an impaired synaptic transmission in the outer plexiform layer (OPL) of the retina. To investigate the basis for this ocular phenotype, we **analyzed** the distribution of beta-**dystroglycan**, a central member of the dystrophin-glycoprotein complex, in the chick retina by using the 43DAG/8D5 **monoclonal antibody**. This **antibody** reacted specifically with chick beta-dystroglycan, as indicated by its staining of the neuromuscular junction, and its reactivity with a single 43-kilodalton band in Western blots. In the retina, beta-dystroglycan was highly concentrated in the OPL and at the vitreal border of the retina, around the inner limiting membrane. Mechanically isolated and flat-mounted inner limiting membranes were stained by the anti-beta-dystroglycan **antibody**, and this immunoreactivity could be extracted by detergent, indicating that beta-dystroglycan is associated with membranous structures bound to the basal lamina. Consistently, electron microscopy showed a concentration of beta-dystroglycan in the endfeet of Muller glial **cells** exclusively in the region of direct contact to the inner limiting membrane. In the OPL, beta-dystroglycan immunoreactivity was concentrated in the distal extensions of rod and cone terminals protruding into the outer plexiform layer. There, beta-dystroglycan codistributed with the alphalbeta subunit of the N-type voltage-gated calcium channel. By contrast to previous reports, we did not **detect** beta-**dystroglycan** directly associated with the synaptic regions of conventional or ribbon synapses of the retina. These results show that in the retina beta-dystroglycan is exclusively expressed by photoreceptors and glial **cells** and that beta-dystroglycan is highly concentrated in subcellular regions of glial **cell** endfeet and photoreceptor terminals. Moreover, the colocalization of beta-dystroglycan with N-type calcium channels in the outer plexiform layer indicates that both proteins might be part of a macromolecular complex.

L70 ANSWER 5 OF 22 MEDLINE
AN 97354185 MEDLINE
DN 97354185 PubMed ID: 9210479
TI The N-terminal region of alpha-dystroglycan is an autonomous globular domain.
AU Brancaccio A; Schulthess T; Gesemann M; Engel J
CS Department of Biophysical Chemistry, Biozentrum, University of Basel, Switzerland.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1997 May 15) 246 (1) 166-72.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-X86073
EM 199708
ED Entered STN: 19970813
Last Updated on STN: 19980206
Entered Medline: 19970804
AB The structure of the N-terminal region of mouse alpha-dystroglycan (DGN) was investigated by expression of two protein **fragments** (residues 30-180 and 30-438) in Escherichia coli **cells**. Trypsin susceptibility experiments show the presence of a stable alpha-dystroglycan N-terminal region (approximately from residue 30 to 315). In addition, guanidinium hydrochloride (Gdn/HCl) denaturation of DGN-(30-438)-peptide, monitored by means of tryptophan fluorescence, produces a cooperative transition typical of folded protein structures.

These results strongly suggest that the alpha-dystroglycan N-terminal is an autonomous folding unit preluding a flexible mucin-like region and that its folding is not influenced by the absence of glycosylation. In order to obtain more information on the structural features of the N-terminal domain we have also used circular dichroism, analytical sedimentation and electron microscopy analysis. Circular dichroic spectra show the absence of typical secondary structure (e.g. alpha-helix or beta-sheet) and closely resemble those recorded for loop-containing proteins. This is consistent with a sequence similarity of the alpha-dystroglycan domain with the loop-containing protein elastase. Analytical ultracentrifugation and electron microscopy analysis reveal that the N-terminal domain has a globular structure. DGN-(30-438)-peptide does not bind in the nanomolar range to an iodinated agrin **fragment** which binds with high affinity to **tissue** purified alpha-**dystroglycan**. No binding was **detected** also to laminin. This result suggests that the alpha-dystroglycan N-terminal domain does not contain the binding site to its extracellular matrix binding partners. It is less likely than the lack of glycosylation reduces its binding affinity, because the N-terminal globular domain only contains two glycosylation sites.

L70 ANSWER 6 OF 22 MEDLINE
AN 96377434 MEDLINE
DN 96377434 PubMed ID: 8783274
TI Dystrophin and the dystrophin-associated glycoprotein, beta-dystroglycan, co-localize in photoreceptor synaptic complexes of the human retina.
AU Drenckhahn D; Holbach M; Ness W; Schmitz F; Anderson L V
CS Institute of Anatomy, University of Wurzburg, Germany.
SO NEUROSCIENCE, (1996 Jul) 73 (2) 605-12.
Journal code: NZR; 7605074. ISSN: 0306-4522.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199703
ED Entered STN: 19970321
Last Updated on STN: 20000303
Entered Medline: 19970311
AB Mutations in the gene encoding for dystrophin, a membrane-associated cytoskeletal protein of muscle and several non-muscle **cells**, are the cause of Duchenne muscular dystrophy and Becker muscular dystrophy. Patients suffering from Duchenne muscular dystrophy have recently been shown to display an abnormal b-wave of the electroretinogram, suggesting that dystrophin is important for normal retinal transmission. In the retina, dystrophin has been localized in the outer plexiform layer where dystrophin co-localizes with postsynaptic markers of photoreceptor synaptic complexes. In the present study we addressed the question of whether two major dystrophin-associated integral membrane proteins of the muscular plasma membrane, beta-dystroglycan and adhalin, are also present in photoreceptor synaptic complexes. By double immunostaining and immunoblotting we show here that beta-dystroglycan is expressed in the human retina where it co-localizes with dystrophin in photoreceptor synaptic complexes most likely on the postsynaptic side. Adhalin was not **detected** in the retina. Since beta-**dystroglycan** is a member of a transmembrane supramolecular complex thought to be important for differentiation of the neuromuscular junction, it is an attractive hypothesis that dystroglycan (linked to dystrophin) might also play a similar role in differentiation of the photoreceptor synapse. A further outcome of this study is that beta-dystroglycan is not only present in the neuromuscular junction but also associated with a well-defined synaptic complex of the central nervous system. These findings indicate a more general role of this dystrophin-associated membrane protein in synaptic functions.

L70 ANSWER 7 OF 22 MEDLINE
AN 96242075 MEDLINE
DN 96242075 PubMed ID: 8656273
TI Neural agrin activates a high-affinity receptor in C2 muscle **cells** that is unresponsive to muscle agrin.
AU Bowen D C; Sugiyama J; Ferns M; Hall Z W
CS Regeneron Pharmaceuticals, Tarrytown, New York 10591, USA.

SO JOURNAL OF NEUROSCIENCE, (1996 Jun 15) 16 (12) 3791-7.
Journal code: JDF; 8102140. ISSN: 0270-6474.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199607
ED Entered STN: 19960808
Last Updated on STN: 19970203
Entered Medline: 19960730
AB During synaptogenesis, agrin, released by motor nerves, causes the clustering of acetylcholine receptors (AChRs) in the skeletal muscle membrane. Although muscle alpha-dystroglycan has been postulated to be the receptor for the activity of agrin, previous experiments have revealed a discrepancy between the biological activity of soluble **fragments** of two isoforms of agrin produced by nerves and muscles, respectively, and their ability to bind alpha-**dystroglycan**. We have **determined** the specificity of the signaling receptor by investigating whether muscle agrin can block the activity of neural agrin on intact C2 myotubes. We find that a large excess of muscle agrin failed to inhibit either the number of AChR clusters or the phosphorylation of the AChR induced by picomolar concentrations of neural agrin. These results indicate that neural, but not muscle, agrin interacts with the signaling receptor. Muscle agrin did block the binding of neural agrin to isolated alpha-dystroglycan, however, suggesting either that alpha-dystroglycan is not the signaling receptor or that its properties in the membrane are altered. Direct assay of the binding of muscle or neural agrin to intact myotubes revealed only low-affinity binding. We conclude that the signaling receptor for agrin is a high-affinity receptor that is highly specific for the neural form.

L70 ANSWER 8 OF 22 MEDLINE
AN 96217379 MEDLINE
DN 96217379 PubMed ID: 8632169
TI Beta-dystroglycan: subcellular localisation in rat brain and detection of a novel immunologically related, postsynaptic density-enriched protein.
AU Mummery R; Sessay A; Lai F A; Beesley P W
CS Division of Biochemistry, School of Biological Sciences, University of London, Egham, Surrey, England.
SO JOURNAL OF NEUROCHEMISTRY, (1996 Jun) 66 (6) 2455-9.
Journal code: JAV; 2985190R. ISSN: 0022-3042.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199607
ED Entered STN: 19960715
Last Updated on STN: 19960715
Entered Medline: 19960701
AB The distribution of a glycoprotein component of the muscle dystrophin complex, beta-**dystroglycan**, has been **determined** in subcellular fractions of adult rat forebrain. The results show that beta-dystroglycan is enriched in several membrane fractions, including synaptic membranes, but in marked contrast to dystrophin is not detectable in the postsynaptic density fraction. The antiserum also recognises a second molecular species of apparent molecular mass of 164 kDa which is highly enriched in the postsynaptic density fraction. Preabsorption of the antiserum with the antigen (a 22-mer peptide corresponding to the C-terminal sequence of rabbit skeletal muscle beta-dystroglycan) abolished reactivity against both beta-dystroglycan and the 164-kDa postsynaptic density-enriched protein, confirming that the two species are immunologically related. Enzymatic removal of N-linked oligosaccharide lowered the apparent molecular mass of beta-dystroglycan by 3 kDa but did not alter the mass of the 164-kDa species.

L70 ANSWER 9 OF 22 MEDLINE
AN 96197945 MEDLINE
DN 96197945 PubMed ID: 8967753
TI Ultrastructural localization of adhalin in normal murine skeletal myofiber.

AU Wakayama Y; Inoue M; Murahashi M; Shibuya S; Jimi T; Kojima H; Oniki H
CS Division of Neurology, Department of Medicine, Showa University Fujigaoka Hospital, Yokohama, Japan.
SO ANNALS OF NEUROLOGY, (1996 Feb) 39 (2) 217-23.
Journal code: 6AE; 7707449. ISSN: 0364-5134.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199612
ED Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961212
AB The ultrastructural localization of adhalin and its relations to dystrophin, beta-dystroglycan, and beta-spectrin were studied in normal murine skeletal myofibers. The C-terminal peptides of adhalin and beta-dystroglycan were synthesized based on their cDNAs, and the affinity-purified **antibodies** against these peptides were produced. Single-immunolabeling electron microscopy showed that the adhalin was located just inside the muscle plasma membrane or inside the myofiber a short distance from the plasma membrane. The adhalin signal was also noted at the sarcoplasmic side of plasmalemmal invaginations or at vesicular structures in subsarcolemmal areas. Double-immunogold-labeling electron microscopy disclosed a similar localization of dystrophin, beta-dystroglycan, and beta-spectrin. The close association of adhalin with dystrophin or beta-dystroglycan was demonstrated by formation of doublets by signals of **antibodies** of adhalin with those of dystrophin or beta-**dystroglycan** and was confirmed by statistical **analyses**. This study demonstrated that the location of adhalin is close to that of dystrophin and beta-dystroglycan at the muscle plasma membrane.

L70 ANSWER 10 OF 22 MEDLINE
AN 95310367 MEDLINE
DN 95310367 PubMed ID: 7790379
TI Non-muscle alpha-dystroglycan is involved in **epithelial** development.
AU Durbeej M; Larsson E; Ibraghimov-Beskrovnaya O; Roberds S L; Campbell K P; Ekblom P
CS Department of Animal Physiology, Uppsala University, Sweden.
SO JOURNAL OF CELL BIOLOGY, (1995 Jul) 130 (1) 79-91.
Journal code: HMV; 0375356. ISSN: 0021-9525.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199507
ED Entered STN: 19950807
Last Updated on STN: 19950807
Entered Medline: 19950727
AB The dystroglycan complex is a transmembrane linkage between the cytoskeleton and the basement membrane in muscle. One of the components of the complex, alpha-dystroglycan binds both laminin of muscle (laminin-2) and agrin of muscle basement membranes. **Dystroglycan** has been **detected** in nonmuscle **tissues** as well, but the physiological role in nonmuscle **tissues** has remained unknown. Here we show that dystroglycan during mouse development in nonmuscle **tissues** is expressed in **epithelium**. In situ hybridization revealed strong expression of dystroglycan mRNA in all studied **epithelial** sheets, but not in endothelium or mesenchyme. Conversion of mesenchyme to **epithelium** occurs during kidney development, and the embryonic kidney was used to study the role of alpha-dystroglycan for **epithelial** differentiation. During in vitro culture of the metanephric mesenchyme, the first morphological signs of **epithelial** differentiation can be seen on day two. Northern blots revealed a clear increase in dystroglycan mRNA on day two of in vitro development. A similar increase of expression on day two was previously shown for laminin alpha 1 chain. Immunofluorescence showed that dystroglycan is strictly located on the basal side of developing kidney **epithelial cells**. **Monoclonal**

antibodies known to block binding of alpha-dystroglycan to laminin-1 perturbed development of **epithelium** in kidney organ culture, whereas control **antibodies** did not do so. We suggest that the dystroglycan complex acts as a receptor for basement membrane components during **epithelial** morphogenesis. It is likely that this involves binding of alpha-dystroglycan to E3 **fragment** of laminin-1.

L70 ANSWER 11 OF 22 MEDLINE
AN 94265258 MEDLINE
DN 94265258 PubMed ID: 8205617
TI Dystroglycan-alpha, a dystrophin-associated glycoprotein, is a functional agrin receptor.
AU Gee S H; Montanaro F; Lindenbaum M H; Carbonetto S
CS Centre for Research in Neuroscience, McGill University, Montreal General Hospital Research Institute, Quebec, Canada.
SO CELL, (1994 Jun 3) 77 (5) 675-86.
Journal code: CQ4; 0413066. ISSN: 0092-8674.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199407
ED Entered STN: 19940721
Last Updated on STN: 19940721
Entered Medline: 19940711
AB Aggregation of acetylcholine receptors (AChRs) on skeletal muscle fibers is thought to be mediated by the basal lamina protein agrin. Structural similarities shared by agrin and laminin suggested that the laminin receptor dystroglycan-alpha, part of a dystrophin-receptor complex, might also bind agrin. We show here that dystroglycan-alpha and dystrophin-related protein (DRP/utrophin) are concentrated within AChR aggregates in cultures of C2 myotubes and that agrin binds specifically to **dystroglycan**-alpha in in vitro **assays**. This binding is calcium dependent and is inhibited by **monoclonal antibody** (MAb) IIH6 against dystroglycan-alpha, heparin, and laminin, but not by fibronectin. In S27 **cells**, which do not aggregate AChRs spontaneously, agrin and laminin binding to dystroglycan-alpha are dramatically decreased. Moreover, MAb IIH6 significantly inhibits agrin-induced AChR aggregation on C2 **cells**. We conclude that dystroglycan-alpha is an agrin-binding protein and part of a dystrophin-receptor complex involved in AChR aggregation.

L70 ANSWER 12 OF 22 BIOSIS COPYRIGHT 2001 BIOSIS
AN 2001:339903 BIOSIS
DN PREV200100339903
TI Dystroglycan distribution in adult mouse brain: A light and electron microscopy study.
AU Zaccaria, M. L.; Di Tommaso, F.; Brancaccio, A.; Paggi, P.; Petrucci, T. C. (1)
CS (1) Laboratorio di Biologia Cellulare, Istituto Superiore di Sanita, 00161, Rome: tcpetru@iss.it Italy
SO Neuroscience, (10 May, 2001) Vol. 104, No. 2, pp. 311-324. print.
ISSN: 0306-4522.
DT Article
LA English
SL English
AB Dystroglycan, originally identified in muscle as a component of the dystrophin-associated glycoprotein complex, is a ubiquitously expressed **cell**-surface receptor that forms a transmembrane link between the extracellular matrix and the cytoskeleton. It contains two subunits, alpha and beta, formed by proteolytic cleavage of a common precursor. In the brain, different neuronal subtypes and glial **cells** may express dystroglycan in complex with distinct cytoplasmic proteins such as dystrophin, utrophin and their truncated forms. To examine the distribution of dystroglycan in adult mouse brain, we raised **antibodies** against the recombinant amino- and carboxyl-terminal domains of alpha-dystroglycan. On western blot, the **antibodies** recognized specifically alpha-dystroglycan in cerebellar extracts. Using light microscopy, alpha-dystroglycan was found in neurons of the cerebral

cortex, hippocampus, olfactory bulb, basal ganglia, thalamus, hypothalamus, brainstem and cerebellum, where dystrophin and its truncated isoforms are also known to be present. Electron microscopy revealed that alpha-dystroglycan immunoreactivity was preferentially associated with the postsynaptic specializations. **Dystroglycan** immunostaining was also **detected** in perivascular astrocytes and in those facing the pia mater, where utrophin and dystrophin truncated isoforms are present. The **cell** body and endfeet of astrocytes around **blood** vessels and the endothelial **cells** at the **blood**-brain barrier also expressed dystroglycan. From these data, we suggest that dystroglycan, by bridging the extracellular matrix and the cytoskeleton, may play an important functional role at specialized intercellular contacts, synapses and the **blood**-brain barrier, whose structural and functional organization strictly depend on the integrity of the extracellular matrix-cytoskeleton linkage.

L70 ANSWER 13 OF 22 BIOSIS COPYRIGHT 2001 BIOSIS
AN 2001:65833 BIOSIS
DN PREV200100065833
TI Role of acetylcholine receptors in agrin-induced clustering of postsynaptic proteins.
AU Marangi, A. P. (1); Mittaud, P.; Moransard, M.; Erb-Voegtli, S.; Fuhrer, C.
CS (1) University of Zuerich, Zuerich Switzerland
SO Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-26.12. print.
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000 Society for Neuroscience . ISSN: 0190-5295.
DT Conference
LA English
SL English
AB During synaptogenesis at the neuromuscular junction, agrin causes clustering of acetylcholine receptors (AChRs) and other proteins via MuSK and rapsyn. We have investigated the role of the AChR itself in agrin-induced protein aggregation, using two mutant derivatives of C2 lacking its alpha subunit. Immunoblot analysis showed that the amounts of most other postsynaptic components are normal in these **cells**, apart from a reduction in beta AChRs and rapsyn. Immunofluorescence microscopy revealed that alpha-dystrobrevin, MuSK and utrophin are clustered by agrin in mutant **cells**, whereas no aggregates were **detected** for rapsyn, alpha-**dystroglycan**, beta-**dystroglycan** and syntrophin, unlike in C2 **cells**, where all proteins analysed co-clustered with AChRs. In the mutants, MuSK was activated normally by agrin, as shown by MuSK immunoprecipitation and phosphotyrosine immunoblotting. Independently, C2 myotubes were treated with anti alpha-AChR **antibodies**, which resulted in reduced amounts of AChRs, without substantially affecting other proteins, and abolished AChR clustering. Under these circumstances, rapsyn was not clustered by agrin but colocalized with remaining AChRs in microaggregates, and the distribution of other postsynaptic proteins was the same as in the mutant **cells**. These results indicate that a subset of postsynaptic proteins clusters independently of the AChR, whereas others require the presence of AChRs, thus establishing a hierarchy of clustering, in which AChRs are necessary to link together a full set of postsynaptic components and play an active role in postsynaptic organization.

L70 ANSWER 14 OF 22 BIOSIS COPYRIGHT 2001 BIOSIS
AN 2000:48848 BIOSIS
DN PREV200000048848
TI Structural and functional analysis of the N-terminal extracellular region of beta-dystroglycan.
AU Di Stasio, Enrico; Sciandra, Francesca; Maras, Bruno; Di Tommaso, Francesca; Petrucci, Tamara C.; Giardina, Bruno; Brancaccio, Andrea (1)
CS (1) Centro di Studio per la Chimica dei Recettori e delle Molecole Biologicamente Attive (CNR), Istituto di Chimica e Chimica Clinica, Universita Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168, Rome Italy
SO Biochemical and Biophysical Research Communications, (Dec. 9, 1999) Vol.

266, No. 1, pp. 274-278.
ISSN: 0006-291X.
DT Article
LA English
SL English
AB A protein **fragment** corresponding to the mouse beta-dystroglycan N-terminal extracellular region from position 654 to 750, beta-DG(654-750) was recombinantly expressed in BL21(DE3) Escherichia coli **cells**. Secondary structure prediction of the protein **fragment** reveals about 70% of random coil, as confirmed by circular dichroism analysis. Moreover, fluorescence analysis shows that the tryptophan residue in position 659 lays in a solvent-exposed fashion. These data suggest that the beta-DG(654-750) is likely to have a quite flexible structure and to be only partially folded. Interestingly, the protein still retains its biological function since using solid-phase assays we have detected binding of biotinylated beta-DG(654-750) both to native alpha-dystroglycan and to a recombinant **fragment** which spans the C-terminal region of alpha-dystroglycan.

L70 ANSWER 15 OF 22 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1999:256134 BIOSIS
DN PREV199900256134
TI Adhesion of cultured bovine aortic endothelial **cells** to laminin-1 mediated by dystroglycan.
AU Shimizu, Hisao; Hosokawa, Hiroshi; Ninomiya, Haruaki; Miner, Jeffrey H.; Masaki, Tomoh (1)
CS (1) National Cardiovascular Research Institute, 5-7-1 Fujishirodai, Suita, Osaka, 565-8565 Japan
SO Journal of Biological Chemistry, (April 23, 1999) Vol. 274, No. 17, pp. 11995-12000.
ISSN: 0021-9258.
DT Article
LA English
SL English
AB Expression of dystroglycan (DG) by cultured bovine aortic endothelial (BAE) **cells** was confirmed by cDNA cloning from a BAE cDNA library, Northern blotting of mRNA, Western blotting of membrane proteins, and double immunostaining with **antibodies** against betaDG and platelet endothelial **cell** adhesion molecule-1. Immunocytochemical analysis revealed localization of DG in multiple plaques on the basal side of resting **cells**. This patchy distribution was obscured in migrating **cells**, in which the most prominent staining was observed in the trailing edge anchoring the **cells** to the substratum. Biotin-labeled laminin-1 overlay assay of dissociated BAE membrane proteins indicated the interaction of laminin-1 with alphaDG. The laminin alpha5 globular domain **fragment** expressed in bacteria and labeled with biotin could also bind alphaDG on the membrane blot, and the unlabeled **fragment** disrupted the binding of biotin-laminin-1 to alphaDG. The interaction of biotin-laminin-1 with alphaDG was inhibited by soluble alphaDG contained in the conditioned medium from DG cDNA-transfected BAE **cells** and by a series of glycosaminoglycans (heparin, dextran sulfate, and fucoidan). Soluble alphaDG in the conditioned medium inhibited the adhesion of BAE **cells** to laminin-1-coated dishes, whereas it had no effect on their adhesion to fibronectin. All three glycosaminoglycans that disrupted the biotin-laminin-1 binding to alphaDG inhibited BAE **cell** adhesion to laminin-1, whereas they failed to inhibit the adhesion to fibronectin. These results indicate a role of DG as a non-integrin laminin receptor involved in vascular endothelial **cell** adhesion to the extracellular matrix.

L70 ANSWER 16 OF 22 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1996:189866 BIOSIS
DN PREV199698745995
TI Ultrastructural localization of adhalin in normal murine skeletal myofiber.
AU Wakayama, Yoshihiro (1); Inoue, Masahiko; Murahashi, Makoto; Shibuya, Seiji; Jimi, Takahiro; Kojima, Hiroko; Oniki, Hiroaki
CS (1) Division Neurology, Department Medicine, Showa University Fujigaoka Hospital, 1-30, Fujigaoka, Aoba-ku, Yokohama 227 Japan

SO Annals of Neurology, (1996) Vol. 39, No. 2, pp. 217-223.
ISSN: 0364-5134.
DT Article
LA English
AB The ultrastructural localization of adhalin and its relations to dystrophin, beta-dystroglycan, and beta-spectrin were studied in normal murine skeletal myofibers. The C-terminal peptides of adhalin and beta-dystroglycan were synthesized based on their cDNAs, and the affinity-purified **antibodies** against these peptides were produced. Single-immunolabeling electron microscopy showed that the adhalin was located just inside the muscle plasma membrane or inside the myofiber a short distance from the plasma membrane. The adhalin signal was also noted at the sarcoplasmic side of plasmalemmal invaginations or at vesicular structures in subsarcolemmal areas. Double-immunogold-labeling electron microscopy disclosed a similar localization of dystrophin, beta-dystroglycan, and beta-spectrin. The close association of adhalin with dystrophin or beta-dystroglycan was demonstrated by formation of doublets by signals of **antibodies** of adhalin with those of dystrophin or beta-**dystroglycan** and was confirmed by statistical **analyses**. This study demonstrated that the location of adhalin is close to that of dystrophin and beta-dystroglycan at the muscle plasma membrane.

L70 ANSWER 17 OF 22 USPATFULL
AN 2001:44435 USPATFULL
TI Sarcospan-deficient mouse as a model for clinical disorders associated with sarcospan mutations
IN Campbell, Kevin P., Iowa City, IA, United States
Lebakken, Connie, Iowa City, IA, United States
Crosbie, Rachelle, Iowa City, IA, United States
Williamson, Roger, Iowa City, IA, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
PI US 6207878 B1 20010327
AI US 1999-422762 19991021 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Priebe, Scott D.; Assistant Examiner: Shukla, Ram R.
LREP Farrell, Kevin M.
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1173
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a transgenic knockout mouse whose genome has a homozygous disruption in its endogenous sarcospan gene, wherein the disruption prevents the synthesis of functional sarcospan in **cells** of the mouse. The mouse is characterized as exhibiting from 1.4 to 6.8 fold larger epididymal fat pad deposits as compared to the epididymal fat pad deposits of a wild type mouse. Methods for production of the mouse are presented. Also disclosed are **cells** derived from the transgenic knockout mouse. The mouse can be used in a method for identifying therapeutic agents for the treatment of an individual diagnosed with a metabolic disorder associated with a reduction or loss of expression of wild-type sarcospan. An example of such a disorder is weight gain in the individual associated with a reduction or loss of expression of wild-type sarcospan. These specific methods are also provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L70 ANSWER 18 OF 22 USPATFULL
AN 2001:37014 USPATFULL
TI Pathogenesis of cardiomyopathy
IN Campbell, Kevin P., Iowa City, IA, United States
Coral, Ramon, Iowa City, IA, United States
Cohn, Ronald, Iowa City, IA, United States
Williamson, Roger, Iowa City, IA, United States
Durbeej, Madeleine, Iowa City, IA, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States

(U.S. corporation)
PI US 6201168 B1 20010313
AI US 1999-378418 19990820 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Hauda, Karen M.; Assistant Examiner: Shukla, Ram R.
LREP Farrell, Kevin M.
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 1700
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a mouse, **cells** derived therefrom, and methods for using the mouse, the mouse being homozygous for a disrupted .delta.-sarcoglycan gene, the disruption in the gene having been introduced into the mouse or an ancestor of the mouse at an embryonic stage. The disruption prevents the synthesis of functional .delta.-sarcoglycan in **cells** of the mouse and results in the mouse having a reduced amount of .beta.- and .epsilon.-sarcoglycan and sarcospan, and a disruption of the sarcoglycan-sarcospan complex in smooth muscle of the mouse. Also disclosed is a mouse, **cells** derived therefrom, and methods for using the mouse, the mouse being homozygous for a disrupted .beta.-sarcoglycan gene, the disruption in the gene having been introduced into the mouse or an ancestor of the mouse at an embryonic stage. The disruption prevents the synthesis of functional .beta.-sarcoglycan in **cells** of the mouse and results in the mouse having a reduced amount of .delta.-and .epsilon.-sarcoglycan and sarcospan and .alpha.-dystroglycan in smooth muscle of the mouse.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L70 ANSWER 19 OF 22 USPATFULL
AN 2000:84252 USPATFULL
TI Arenavirus receptor and methods of use
IN Campbell, Kevin P., Iowa City, IA, United States
Henry, Michael, Iowa City, IA, United States
Yamada, Hiroki, Iowa City, IA, United States
Williamson, Roger, Iowa City, IA, United States
Cao, Wei, San Diego, CA, United States
Oldstone, Michael, La Jolla, CA, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
PI US 6083911 20000704
AI US 1998-208707 19981210 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Travers, Russell
LREP Farrell, Kevin M.
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 1064
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a method for inhibiting the binding of an arenavirus to a cellular receptor. The method involves providing, in soluble form, a reagent comprising .alpha.-dystroglycan or a portion thereof, the reagent being characterized by the ability to bind to the arenavirus thereby inhibiting the binding of the arenavirus to the cellular receptor. The reagent is contacted with an arenavirus particle prior to infection of a cell by the arenavirus particle. Also disclosed are methods for treating an arenavirus infection in a patient and preventing an arenavirus infection in an individual at risk. These methods involve providing a therapeutic composition comprising .alpha.-dystroglycan or a portion thereof which is characterized by the ability to bind to arenaviruses, thereby inhibiting the binding of arenaviruses to a cellular receptor; and administering the composition to the patient or individual at risk. Arenaviruses to which the methods of the present invention apply include, without limitation, Lymphocyte Choriomeningitis Virus, Lassa fever virus, Mobala, and Oliveros. In another aspect, the

disclosure relates to an embryonic stem cell line, and cells derived therefrom, which is homozygous for a disrupted dystroglycan gene, wherein the disruption prevents the synthesis of functional dystroglycan in the cells. Applications of the dystroglycan null embryonic stem cells include producing dystroglycan or a portion thereof in the cells and also for identifying portions of dystroglycan necessary for arenavirus infection. Also disclosed is a method for identifying antiviral compounds which interfere specifically with the binding of arenavirus and .alpha.-**dystroglycan**, comprising providing a binding **assay** system for the determination of binding of arenavirus and .alpha.-dystroglycan. The candidate antiviral compounds are introduced into the binding assay system and antiviral compounds which substantially inhibit binding of arenavirus to .alpha.-dystroglycan are identified.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L70 ANSWER 20 OF 22 USPATFULL
AN 1999:12758 USPATFULL
TI Merosin deficiency-type congenital muscular dystrophy
IN Campbell, Kevin P., Iowa City, IA, United States
Sunada, Yoshihide, Iowa City, IA, United States
Tome, Fernando M. S., Paris, France
Fardeau, Michel, Sceaux, France
PA University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
PI US 5863743 19990126
AI US 1994-289668 19940812 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Mosher, Mary E.; Assistant Examiner: Wortman, Donna C.
LREP Farrell, Kevin M.
CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN 19 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 645
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a method for aiding in the diagnosis of merosin deficiency-type congenital muscular dystrophy (CMD). The method is based on the discovery of a previously unidentified form of CMD which is characterized by a substantial reduction in the levels of merosin in skeletal muscle **tissue** containing normal levels of dystrophin and dystrophin-associated proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L70 ANSWER 21 OF 22 USPATFULL
AN 97:104106 USPATFULL
TI Polyclonal and **monoclonal antibodies** against a 43 KDA dystrophin associated protein
IN Campbell, Kevin P., Iowa City, IA, United States
Ibraghimov, Oxana B., Southboro, MA, United States
Ervasti, James M., Middleton, WI, United States
Leveille, Cynthia J., Iowa City, IA, United States
PA The University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
PI US 5686073 19971111
AI US 1995-483278 19950607 (8)
RLI Continuation-in-part of Ser. No. US 1993-123161, filed on 16 Sep 1993, now patented, Pat. No. US 5449616 which is a continuation-in-part of Ser. No. US 1992-946234, filed on 14 Sep 1992, now patented, Pat. No. US 5308752 which is a continuation-in-part of Ser. No. US 1992-841654, filed on 20 Feb 1992, now patented, Pat. No. US 5260209 which is a continuation-in-part of Ser. No. US 1990-527583, filed on 23 May 1990, now patented, Pat. No. US 5187063
DT Utility
FS Granted
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: Vander Vegt, F. Pierre
LREP Farrell, Kevin M.

CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 2663
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods for the preparation of polyclonal and **monoclonal antibodies** which bind specifically to a 43 kDa dystrophin-associated. The molecular weight of the 43 kDa protein is determined by electrophoretic separation under denaturing conditions, followed by transfer to a solid support and staining with wheat germ agglutinin. The method includes a step in which the peptide PKNMTPYRSPPPYVP (SEQ ID NO: 15) is administered to stimulate an immune response. Also disclosed are polyclonal and **monoclonal antibodies** which bind specifically to the 43 kDa dystrophin-associated protein.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L70 ANSWER 22 OF 22 USPATFULL
AN 95:82215 USPATFULL
TI Nucleic acid encoding dystrophin-associated protein
IN Campbell, Kevin P., Iowa City, IA, United States
Roberds, Steven L., Iowa City, IA, United States
Anderson, Richard D., Coralville, IA, United States
PA University of Iowa Research Foundation, Iowa City, IA, United States (U.S. corporation)
PI US 5449616 19950912
AI US 1993-123161 19930916 (8)
RLI Continuation-in-part of Ser. No. US 1992-946234, filed on 14 Sep 1992, now patented, Pat. No. US 5308752 which is a continuation-in-part of Ser. No. US 1992-841654, filed on 20 Feb 1992, now patented, Pat. No. US 5260209 which is a continuation-in-part of Ser. No. US 1990-527583, filed on 23 May 1990, now patented, Pat. No. US 5187063
DT Utility
FS Granted
EXNAM Primary Examiner: Patterson, Jr., Charles L.; Assistant Examiner: Jacobson, Dian C.
LREP Farrell, Kevin M.
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 2676
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are nucleic acid sequences encoding components of the dystrophin-glycoprotein complex. The components include dystroglycan, the 50 kDa protein component and the 59 kDa protein component. Also disclosed are compositions and methods which relate to the disclosed sequences.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d kwic 22

L70 ANSWER 22 OF 22 USPATFULL
SUMM Dystrophin has been shown to be associated with a large oligomeric complex of sarcolemmal glycoproteins (see, e.g., Ervasti and Campbell, **Cell** 66: 1121-1131 (1991)). Substantial reduction in selected components of the dystrophin-glycoprotein complex have also been found to correlate with disease. . .
SUMM . . . protein and the 59 kDa dystrophin-associated protein. Related to this are DNA expression constructs which include the nucleic acid, and **cells** transformed with such constructs.
DETD . . . to isolate the dystrophin-glycoprotein complex is known as immunoaffinity purification. This technique utilizes the unique high specificity of polyclonal and **monoclonal antibodies** as well as selected lectins. Such highly specific molecules are extremely valuable tools for rapid, selective purification of antigens. In. . . and this is used to selectively adsorb antigen from a mixture containing many other antigens. The antigens for which the

antibody has no affinity can be washed away, and the purified antigen then eluted from the bound **antibody** or lectin with an elution buffer. Examples of **antibodies** and lectin molecules which are useful for the immunopurification of the dystrophin complex components are described in detail below.

DETD Preparation of **Antibodies** Reactive with Components of the Dystrophin-Glycoprotein Complex

DETD **Monoclonal** and polyclonal **antibodies** specific for non-dystrophin components of the dystrophin-glycoprotein complex are particularly useful in the isolation and diagnostic methods of this invention. **Monoclonal antibodies** useful in this invention are obtained by well known hybridoma methods. An animal is immunized with a preparation containing the dystrophin-glycoprotein complex. A fused **cell** hybrid is then formed between **antibody**-producing **cells** from the immunized animal and an immortalizing **cell** such as a myeloma.

DETD In preferred embodiments, anti-non-dystrophin component **monoclonal antibodies** of this invention are produced by murine hybridomas formed by fusion of: a) mouse myeloma or hybridoma which does not secrete **antibody** with b) murine spleen **cells** which secrete **antibodies** obtained from mice immunized against dystrophin-glycoprotein complex.

DETD . . . injection of dystrophin-glycoprotein complex followed by a number of boosting injections of dystrophin-glycoprotein complex. During or after the immunization procedure, **sera** of the mice may be screened to identify those mice in which a substantial immune response to the complex has been evoked. From selected mice, the spleen **cells** are obtained and fusions are performed. Suitable fusion techniques are the Sendai virus technique (Kohler, G. and Milstein, C., Nature, 256:495 (1975)), or the polyethylene glycol method (Kennet, R. H., "**Monoclonal Antibodies**, Hybridomas-A New Dimension in Biological Analysis, " Eds R. H. Kennet, T. J. McKern and K. B. Bechtol, Plenum Press,. . .

DETD The hybridomas are then screened for production of anti-non-dystrophin component **antibodies**. A suitable screening technique is a solid phase radioimmunoassay. A solid phase immunoadsorbent is prepared by coupling dystrophin-glycoprotein complex or. . . of hybridomas. After a period of incubation, the solid phase is separated from the supernatants, then contacted with a labeled **antibody** against murine immunoglobulin. Label associated with the immunoadsorbent indicates the presence of hybridoma products reactive with dystrophin-glycoprotein complexes or non-dystrophin. . .

DETD The **monoclonal** anti-non-dystrophin component **antibodies** can be produced in large quantities by injecting anti-non-dystrophin component **antibody** producing hybridoma **cells** into the peritoneal cavity of mice and, after an appropriate time, harvesting acites fluid from the mice which yield a high titer of homogenous **antibody**. The **monoclonal antibodies** are isolated therefrom. Alternatively, the **antibodies** can be produced by culturing anti-non-dystrophin component **antibody** producing **cells** in vitro and isolating secreted **monoclonal** anti-non-dystrophin component **antibodies** from the **cell** culture medium directly.

DETD Another method of forming **antibody**-producing **cells** is by viral or oncogenic transformation. For example, a B-lymphocyte which produced a non-dystrophin component specific **antibody** may be infected and transformed with a virus, such as the Epstein-Barr virus, to give an immortal **antibody**- producing **cell** (Kozbon and Roder, Immunol. Today 4: 72-79 (1983)). Alternatively, the B-lymphocyte may be transformed by a transforming gene or gene. . .

DETD Polyclonal **antibodies** can be prepared by immunizing an animal with a crude preparation of the dystrophin-glycoprotein complex or the purified non-dystrophin components of the complex. The animal is maintained under conditions whereby **antibodies** reactive with the components of the complex are produced. **Blood** is collected from the animal upon reaching a desired titer of **antibodies**. The **serum** containing the polyclonal **antibodies** (antisera) is separated from the other **blood** components. The polyclonal **antibody**-containing **serum** can optionally be further separated into fractions of particular types of

antibodies (e.g., IgG or IgM) or monospecific **antibodies** can be affinity purified from polyclonal **antibody** containing **serum**.

DETD . . . quantity of the non-dystrophin components of the dystrophin-glycoprotein complex and affliction by muscular dystrophy. As described in the Exemplification below, **monoclonal** or polyclonal **antibodies** can be used to detect the absence or reduction of a particular non-dystrophin component of the complex. In both mouse and human samples of dystrophic **tissue**, muscular dystrophy can be diagnosed by detecting reduction or absence of non-dystrophin components of the complex.

DETD . . . features with Duchenne's muscular dystrophy (DMD) including, for example, mode of onset, rapid progression, hypertrophy of calves and extremely high **serum** creatine kinase levels during the initial stages of the disease (see, e.g., Ben Hamida et al., J. Neurol Sci. 1.07:. . .

DETD . . . the invention, a muscle biopsy sample is treated in a procedure which renders the non-dystrophin components available for complexing with **antibodies** directed against said components. Muscle samples are obtained from patients by surgical biopsy. The site of biopsy could be any. . .

DETD For biopsy samples greater than 500 mg, the muscle **tissue** can be homogenized by mechanical disruption using apparatus such as a hand operated or motor driven glass homogenizer, a Waring. . . PMSF (40 .mu.g/ml). Heavy microsomes can be prepared from homogenized skeletal muscle by the method of Mitchel, et al. (J. **Cell**. Biol., 95: 1008-1016 (1983)). The microsomes are then washed with a physiological salt solution and solubilized in saline containing detergent. . .

DETD . . . proteins can be accomplished by the use of general protein dyes such as Amido black or Coomassie brilliant blue. Alternatively, **antibodies** which are specific for the known non-dystrophin components of the dystrophin-glycoprotein complex can be labeled with a detectable reporter group. . . components. An example of this method is the well known Western blot method. In yet another alternative detection method, unlabeled **antibodies** specific for a component of the dystrophin-glycoprotein complex are incubated with a muscle **tissue** sample under conditions appropriate for binding. The specific binding of these **antibodies** to the muscle **tissue** sample is detected through the use of labeled secondary **antibodies** by conventional techniques.

DETD Alternatively, **tissue** specimens (e.g., human biopsy samples) can be tested for the presence of the components of the dystrophin-glycoprotein complex by using **monoclonal** or polyclonal **antibodies** in an immunohistochemical technique, such as the immunoperoxidase staining procedure. In addition, immunofluorescent techniques can be used to examine human **tissue** specimens. In a typical protocol, slides containing cryostat sections of frozen, unfixed **tissue** biopsy samples are air-dried and then incubated with the anti-non-dystrophin component **antibody** preparation in a humidified chamber at room temperature. The slides are layered with a preparation of fluorescently labeled **antibody** directed against the **monoclonal antibody**. As mentioned above, labeled secondary **antibodies** are also useful for detection. The staining pattern and intensities within the sample are determined by fluorescent light microscopy.

DETD The **antibodies** of the present invention can also be used in an enzyme-linked immunoadsorbant assay (ELISA) for determining the absence or presence of non-dystrophin components of the dystrophin-glycoprotein complex. **Antibodies** against non-dystrophin components to be measured are adsorbed to a solid support, in most cases a polystyrene microtiter plate. After coating the support with **antibody** and washing, a solubilized sample is added. If a non-dystrophin component is present for which the **antibodies** are specific, they will bind to the adsorbed **antibodies**. Next, a conjugate that will also bind to the non-dystrophin component is added. Conjugates are secondary **antibody** molecules to which an enzyme is covalently bound. After addition of a chromogenic substrate for the enzyme, the intensity of. .

DETD In another embodiment of the diagnostic method, **antibodies** specifically reactive with an extracellular component of the

dystrophin-glycoprotein complex (e.g., the 156 kDa component) are labeled with a detectable. . . dystrophy using conventional immunodiagnostic methods. The extracellular components of the dystrophin-glycoprotein complex are exposed on the surface of an intact **cell** and therefore, are reactive with labeled circulating **antibodies** which have the ability to pass through capillary membranes to reach the muscle **tissue** surface. Thus, the disruption of the **cell** is not necessary for diagnosis.

DETD **Antibodies** reactive with dystrophin-associated proteins can be used to isolate and purify nucleic acid which encodes the proteins. This can be accomplished in a variety of ways. For example, monospecific polyclonal **antibodies**, or **monoclonal** **antibodies**, can be used in affinity purification methods to isolate highly purified preparations of individual dystrophin-associated glycoproteins (DAGs). Using standard biochemical. . . probes can be designed and synthesized. Such probes can be used to screen nucleic acid libraries (e.g., cDNA libraries from **tissues** known to express the dystrophin-associated proteins). DNA sequences identified by such a screening method can be used to isolate overlapping. . .

DETD Alternatively, **monoclonal** or polyclonal **antibodies** can be used to screen an expression library, such as a cDNA library prepared in the vector .lambda.gt11. The .lambda.gt11 system enables the expression of DNA **fragments** from a DNA library of interest (e.g., a human genomic DNA library) as a beta-galactosidase fusion protein. Recombinant phage are. . .

DETD . . . peptide portions of same, can be used for example, as a source of highly pure immunogen for the generation of **antibodies** specific to components of the complex. Immunogenic peptides can also be produced synthetically from the known DNA sequence. Alternatively, as. . .

DETD In studies of muscle **tissue** from patients afflicted with severe childhood autosomal recessive muscular dystrophy (SCARMD), it was determined that the 50 kDa dystrophin-associated protein. . . protein was determined to be present at a somewhat reduced level, although not reduced to the degree observed in DMD **tissue**.

DETD . . . an expression vector containing the DNA sequence encoding the human 50 kDa dystrophin-associated glycoprotein can be introduced into the muscle **tissue** of an individual afflicted with SCARMD.

DETD The DNA expression vector can be introduced into the individual in a variety of ways. For example, myoblast **cells** can be isolated from the afflicted individual by biopsy, transformed with the expression construct in vitro and reintroduced to the. . .

DETD A variety of techniques can be employed to ensure that the DNA encoding the dystrophin-associated protein is taken up by **cells** following intramuscular injection. For example, the expression vector employed can be a defective animal virus having the ability to infect human **cells** (e.g., adenovirus or retrovirus derivatives). In addition, liposome technology has been developed in which the expression construct is encapsulated in a membrane having the ability to fuse with mammalian **cell** membranes thereby allowing the transfer of the liposome contents into the mammalian **cell**. Liposomes can be targeted to muscle **cells** specifically through the use of specific membrane markers.

DETD . . . afflicted individual may also offer a viable therapeutic alternative. Because of the difficulties associated with introducing a protein into a **cell** across the **cell** membrane, this therapeutic approach is would be most useful for muscular dystrophies characterized by the absence or reduction in abundance. . .

DETD . . . to be a high affinity laminin binding protein. Although the mechanism underlying the fact that dystrophin deficiency causes the muscle **cell** necrosis characteristic of muscular dystrophy is unknown, experiments suggest that dystrophin functions to link the subsarcolemmal membrane cytoskeleton through a. . . to an extracellular glycoprotein which binds laminin. Although the exact function of the 156 kDa protein is not known, muscle **cells** do interact with the extracellular matrix via specific **cell** surface receptors and thus it is likely that the 156 kDa protein is involved in the interactions between sarcolemma and. . .

DETD . . . a laminin binding domain thereof, would be administered (preferably intravenously) to an afflicted individual. At the surface of

the muscles **tissue**, the 156 kDa protein, or the laminin binding portion thereof, is expected to interact with the sarcolemma and extracellular matrix thereby stabilizing the **tissue**. The progress of therapy can be monitored, for example, by a combination of muscle strength measurement and muscle biopsy analysis.

DETD Heavy microsomes were prepared from rabbit skeletal muscle by the method described in Mitchell, et al. (J. **Cell**. Biol. 95: 1008-1016 (1983)). The microsomes were washed twice with 0.6M KCl in 50 mM tris-HCl, pH 7.4, 0.165M sucrose,. . .

DETD . . . Blue (300 .mu.l of fractions concentrated to 50 .mu.l with a centricon-100) or transferred to nitrocellulose and stained with various **antibodies**. Gel lanes were scanned with a Hoefer GS300 scanning densitometer and analyzed using GS-360 data analysis software.

DETD . . . were immunized with rabbit skeletal muscle membranes and boosted with WGA eluate as described in Jorgensen, A. O., et al., **Cell** Motility and Cytoskeleton, 9: 164-174 (1988).

DETD . . . protein doublet, apparent with Coomassie Blue staining was also stained with concanavalin A. The dystrophin-glycoprotein complex was further characterized with **antibodies** raised against various components of the complex. Antisera from a rabbit which was immunized with a chemically synthesized decapeptide representing. . . to stain a single M.sub.r protein. This protein comigrated with the predominant isoform of dystrophin stained by sheep polyclonal anti-dystrophin **antibodies**.

DETD A library of **monoclonal antibodies** against muscle proteins eluted from WGA-sepharose was also screened for reactivity against components of the dystrophin-glycoprotein complex. Of six hybridomas which showed immuno-fluorescence staining only on the sarcolemma **monoclonal antibodies** XIXC2 and VIA4.sub.2 were found to stain dystrophin on immunoblots. Both dystrophin **monoclonal antibodies** are IgM subtypes, and recognized both native and denatured dystrophin. **Monoclonal antibody** XIXC2 also recognized the minor lower M.sub.r isoform of dystrophin which appears to copurify with the more abundant isoform.

DETD Two of the other sarcolemma-specific **monoclonal antibodies** were specific for components of the dystrophin-glycoprotein complex. The 50 kDa glycoprotein stained with **monoclonal antibody** IVD3.sub.1. **Monoclonal** IVD3.sub.1 recognized only the nonreduced form of the 50 kDa glycoprotein and it is not highly crossreactive. **Monoclonal antibody** VIA4.sub.1 stained the 156 kDa glycoprotein which copurified with dystrophin. **Monoclonal antibody** VIA4.sub.1 recognized the denatured form of the 156 kDa glycoprotein and is highly crossreactive.

DETD . . . 5% normal goat antiserum in phosphate buffered saline, followed by a two hour incubation at 37.degree. C. with the primary **antibody** (hybridoma supernatants or 1:1000 diluted antiserum). After washing in PBS, the sections were further incubated for 30 minutes at 37.degree.. . . or anti-rabbit IgG and subsequently examined in a Leitz fluorescence microscope. Staining of cryostat sections was not observed with non-immune **serum**, nor was there any nonspecific binding to the **tissue** by fluorescein-labeled secondary **antibody**.

DETD The antisera to the C-terminal amino acid sequence of human dystrophin showed immunofluorescence staining only on the **cell** periphery which indicates a restricted localization of dystrophin to the sarcolemma of rabbit skeletal muscle. This observation was confirmed by staining rabbit skeletal muscle with **monoclonal antibody** XIXC2 against dystrophin and, again, localization was observed in the sarcolemma of the rabbit skeletal muscle. The 50 kDa glycoprotein, stained with **monoclonal** IVD3.sub.1, has been localized exclusively to the sarcolemmal membrane of rabbit skeletal muscle. **Monoclonal antibody** VIA4.sub.1 exhibited weak, but specific, immunofluorescent staining of the sarcolemmal membrane consistent with its low affinity for the native 156. . .

DETD . . . dystrophin, the 156 kDa and 50 kDa glycoproteins were retained by the beads and not selectively proteolyzed. Initial experiments with **monoclonal** VIA4.sub.1 (anti-156 kDa glycoprotein) have indicated that it has too low an affinity for the native 156 kDa glycoprotein to. . .

DETD . . . kDa glycoprotein in each preparation was estimated densitometrically from autoradiographs of identical blots incubated with .sup.125 I-labeled sheep anti-mouse secondary **antibody**.

DETD . . . that dystrophin was completely absent from dystrophic mouse membranes. In addition, comparison of normal and dystrophic mouse with immunostaining by **monoclonal antibody** VIA4.sub.1 against the 156 kDa glycoprotein revealed that the 156 kDa glycoprotein was absent or greatly reduced in dystrophic mouse. . . antisera against either the ryanodine receptor, or the dihydropyridine receptor, did not differ between control and dystrophic mouse muscle membranes. **Monoclonal antibody** IVD3.sub.1 against the 50 kDa glycoprotein did not crossreact with normal mouse membranes and, thus, could not be evaluated. The. . . control and dystrophic mice. Estimation of the 156 kDa glycoprotein remaining in the dystrophic muscle membranes using .sup.125 I-labeled secondary **antibodies** and total membrane preparations from four different control and four different dystrophic mice revealed an average reduction of 85% in. . .

DETD The dystrophic samples exhibited no staining with **antibodies** against dystrophin by indirect immunofluorescence microscopy and immunoblotting. In contrast to the normal muscle extract, the 3 DMD samples showed greatly reduced staining for the 156 kDa glycoprotein. On the other hand, identical immunoblots stained with **monoclonal antibodies** against the Ca.sup.2+ - dependent ATPase revealed no difference in the staining intensity between normal and dystrophic muscle samples. Again,. . .

DETD . . . Immobilon-P transfer strips containing individual components of the dystrophin-glycoprotein complex separated by SDS-polyacrylamide gel electrophoresis were used to affinity purify **antibodies** specific of the 156 kDa, 59 kDa, 43 kDa and 35 kDa dystrophin-associated proteins. **Antibodies** to the 50 kDa dystrophin-associated glycoprotein were affinity-purified from antisera obtained by immunizing a guinea pig with SDS polyacrylamide gel. . . kDa dystrophin-associated glycoprotein. Immunoblot staining of skeletal muscle microsomes, sarcolemma and purified dystrophin-glycoprotein complex demonstrated that each of the affinity-purified **antibodies** recognized only proteins of the same molecular weight to which they were raised an affinity purified against. This data suggests that the 156 kDa, 59 kDa, 50 kDa, 43 kDa and 35 kDa dystrophin-associated proteins are not proteolytic **fragments** of larger proteins or dystrophin.

DETD . . . 156 kDa dystrophin-associated glycoprotein relative to dystrophin has not been determined because it stains poorly with Coomassie Blue. Therefore, the **antibody** staining intensity was quantitated from autoradiograms of the immunoblots after incubation with [.sup.125 I]-Protein A and was compared to the. . .

DETD . . . the dystrophin-associated proteins was determined by indirect immunofluorescence labeling of transverse cryostat sections of rabbit skeletal muscle. The affinity-purified polyclonal **antibodies** specific for the 156 kDa, 59 kDa, 50 kDa, 43 kDa and 35 kDa dystrophin-associated proteins exhibited immunofluorescent staining of. . . these proteins with the muscle fibre plasma membrane or the intracellular cytoskeleton subjacent to the surface membrane. All five polyclonal **antibodies** against dystrophin-associated proteins illustrated an equal distribution between fast and slow fibers and showed enriched staining at the neuromuscular junction.

DETD . . . that it is the most hydrophobic component of the complex and may explain why we have been unsuccessful in raising **antibodies** to it. It is not clear why the 156 kDa dystrophin-associated glycoprotein was not labeled with [.sup.125 I] TID but. . .

DETD . . . or mAb IVD3.sub.1 (50 kDa glycoprotein)-Sepharose was analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting. Both the dystrophin-and 50 kDa dystrophin-associated glycoprotein-**antibody** matrices were effective in immunoprecipitating greater than 99% of dystrophin and 96% of the 59 kDa, 50 kDa, 43 kDa and 35 kDa dystrophin-associated proteins form untreated dystrophin-glycoprotein complex. Dystrophin- and 50 kDa dystrophin-associated glycoprotein-**antibody** matrices immunoprecipitated 63% and 85% of the 156 kDa dystrophin-associated glycoprotein. The dystrophin-**antibody** matrix immunoprecipitated greater than 99% of the dystrophin from the alkaline-treated dystrophin-associated proteins and only 51% of the 59

kDa. . . dystrophin-associated protein indicating that the interaction between dystrophin and the complex was disrupted by alkaline treatment. The 50 kDa dystrophin-associated glycoprotein-**antibody** matrix immunoprecipitated less than 25%, 32% and 43% of dystrophin-associated proteins from the alkaline-treated complex. However, 96% of the 50. . . kDa, 43 kDa and 35 kDa dystrophin-associated glycoproteins were immunoprecipitated from the alkaline-treated complex using the 50 kDa dystrophin-associated glycoprotein **antibody** matrix. Thus, these data demonstrate that the 50 kDa, 43 kDa and 35 kDa dystrophin-associated proteins alone form a tightly-associated complex. Since the 50 kDa dystrophin-associated glycoprotein-**antibody** matrix immunoprecipitates more of the 156 kDa dystrophin-associated glycoprotein that the dystrophin-**antibody** matrix, these data further suggest that the 156 kDa dystrophin-associated glycoprotein is directly linked to the 50 kDa, 43 kDa. . .

DETD . . . GmbH, Luzern, Switzerland) in the presence of a protease inhibitor cocktail to minimize protein degradation (see Ohlendieck et al., J. **Cell**. Biol. 112: 135-148 (1991)). Homogenates were centrifuged for 15 min at 3,000.times.g and the supernatant filtered through 4 layers of. . . supernatants for 35 min at 140,000.times.g and the final preparation was KCl -washed as described by Ohlendieck et al., J. **Cell** Biol. 112: 135-148 (1991). Cardiac membranes from control and dystrophic dy/dy mice (C57BL/6J-dy; Jackson Laboratory, Bar Harbor, Me.) were prepared. . .

DETD A newly established wheat germ agglutination procedure was employed to isolate purified skeletal muscle sarcolemma (see Ohlendieck et al. J. **Cell** Biol. 112: 135-148 (1991)) and dystrophin-glycoprotein complex was prepared from rabbit skeletal muscle as described by Ervasti et al., (Nature 345: 315-319 (1990)). Protein was determined as described by Peterson (Anal. Biochem. 83: 346-356 (1977)) using bovine **serum** albumin as a standard.

DETD Monospecific **antibodies** against the different components of the dystrophin-glycoprotein complex were produced by injecting the native dystrophin-glycoprotein complex purified as described herein into sheep. After testing the crude sheep antisera for the presence of **antibodies** against the dystrophin-glycoprotein complex, monospecific **antibodies** to 35 kDa glycoprotein, 43 kDa glycoprotein, 50 kDa glycoprotein and 59 kDa protein were affinity purified from individual immobilon. . . components of the dystrophin-glycoprotein complex as described by Sharp et al., (J. Biol. Chem. 264: 2816-2825 (1989)). Specificity of affinity-purified **antibodies** was subsequently determined by immunoblot analysis with rabbit sarcolemma and rabbit dystrophin-glycoprotein complex.

DETD **Monoclonal antibodies** XIXC1 to dystrophin, VIA4.sub.1 to 50 kDa glycoprotein, McB2 to Na/K-ATPase (Urayama et al., J. Biol. Chem. 264: 8271-8280 (1989)) and IID8 to cardiac Ca.sup.2+ -ATPase (Jorgensen et al., **Cell** Motil Cytoskel. 9: 164-174 (1988)) were previously characterized by extensive immunofluorescence and immunoblot analysis (Ohlendieck et al., J. **Cell** Biol. 112: 135-148 (1991)). Rabbit polyclonal **antibodies** against the C-terminal sequences of human dystrophin and human dystrophin-related protein (DRP) were affinity-purified and characterized as described (Ervasti et al., J. Biol. Chem. 266: 9161-9165 (1991)). **Monoclonal antibody** SB-SP-1 against spectrin was purchased from Sigma Chemical Company (St. Louis, Mo.).

DETD . . . were visualized by Coomassie-blue staining and also analyzed by Stainsall staining. Proteins were transferred to nitrocellulose and immunoblot staining with **antibodies** and densitometric scanning was carried out as described above. Both protein A and protein G did not label primary sheep **antibody** sufficiently. Therefore, after primary labeling with sheep **antibody**, immunoblots of mouse muscle membranes were incubated with rabbit anti-sheep secondary **antibody** followed by incubation with .sup.125 I-labeled protein A (Amersham Corporation). This procedure gave reproducibly a very strong signal in autoradiography and enabled densitometric scanning of DAP **antibody** binding to control and mdx mouse muscle membranes.

DETD . . . immunoblots was carried out under optimized conditions as described (Campbell et al., Nature 338: 259-262 (1989); Ohlendieck et al., J. **Cell** Biol. 112: 135-148 (1991)). Blots were incubated

for 1 hr with 1:1,000 diluted peroxidase-labeled wheat germ agglutinin, concanavalin A and. . . Calif.) and developed in 20 mM Tris-Cl, pH 7.5, 200 mM NaCl using 4-chloro-1-napthol as substrate (Jorgensen et al., J. **Cell** Biol. 110: 1173-1185 (1990)).

DETD . . . .mu.m transverse cryosections from control, mdx and dy/dy mouse skeletal muscle was performed as described by Ohlendieck et al. (J. **Cell** Biol. 112: 135-148 (1991)). Following preincubation for 20 min with 5% normal goat antiserum in PBS (50 mM sodium phosphate, pH 7.4, 0.9% NaCl), cryosections were incubated for 1 hr at 37.degree. C. with primary **antibodies** (1:1,000 dilution of crude antisera or 1:100 dilution of hybridoma supernatant or 1:50 dilution of affinity-purified **antibodies**). After extensive washing in PBS the sections were labeled with 1:100 diluted affinity-purified fluorescein-labeled goat anti-mouse IgG or goat anti-rabbit IgG (Boehringer-Mannheim) and subsequently examined in a Zeiss Axioplan fluorescence microscope. In the case of mouse **monoclonal antibodies** used on mouse cryosections, a biotin-streptavidin system was employed for immunodetection. Affinity-purified primary **antibodies** were biotinylated according to the instructions in the commercially available biotinylation kit from Amersham Corporation. Cryosections were incubated with biotinylated primary **antibody** as already described for unlabeled primary **antibody** and subsequently extensively washed in PBS. Finally, sections were fluorescently labeled by incubation with 1:100 diluted affinity-purified fluorescein-conjugated avidin (Sigma. . .

DETD Immunoblot analysis of **antibodies** to dystrophin-associated proteins

DETD Sheep antiserum raised against the native dystrophin-glycoprotein complex was used to affinity-purify monospecific **antibodies** to the individual components of the tightly associated dystrophin-glycoprotein complex. The high specificity of the eluted, affinity-purified **antibodies** was demonstrated by immunoblot. Sheep **antibodies** to 35 kDa glycoprotein, 43 kDa glycoprotein, 50 kDa glycoprotein and 59 kDa protein exhibited strong labeling of their respective antigen in sarcolemma and isolated dystrophin-glycoprotein complex from rabbit skeletal muscle. These results indicate monospecificity of the affinity-purified **antibodies** for the different components of the dystrophin-glycoprotein complex and this is a crucial prerequisite for the characterization of components of the complex in control, mdx and dy/dy muscle. Sheep **antibodies** to 156 kDa glycoprotein did not exhibit strong labeling in immunoblotting and furthermore the affinity-purification of sheep **antibodies** to 156 kDa glycoprotein is complicated due to contaminating **fragments** from degraded dystrophin molecules. We therefore used the already previously characterized **monoclonal antibody** VIA4.sub.1 for the analysis of 156 kDa glycoprotein, which is a highly specific probe and exhibits strong labeling in immunoblotting.

DETD After characterization, the affinity-purified sheep **antibodies** were used in an extensive immunoblot analysis to compare the expression of components of the dystrophin-glycoprotein complex in skeletal muscle. . .

DETD . . . had not been washed with 0.6M KCl, and also with microsomal membranes prepared as described by Ohlendieck et al., J. **Cell**. Biol. 112: 135-148 (1991). These findings indicate that mdx mouse skeletal muscle are not only deficient in dystrophin, but that. . .

DETD . . . abundance making this animal model a very good control for the status of dystrophin-associated proteins in necrotic, but dystrophin-containing muscle **tissue**. Coomassie-blue staining revealed no apparent differences between membranes isolated from control and dy/dy mouse skeletal muscle and the density of dystrophin-related protein is also comparable between both membrane preparations. Most importantly, **antibodies** to the different dystrophin-associated proteins showed approximately equal amounts of these proteins in skeletal muscle membranes from control and dy/dy. . .

DETD Distribution of Dystrophin-Associated Proteins in Normal and Dystrophic Human **Tissue**

DETD The results disclosed in Example 5 demonstrate the absence, or dramatic reduction in the abundance of, dystrophin-associated proteins in **tissue** samples from dystrophic mice. The present example

discloses a similar finding in human **tissue** samples by immunofluorescence microscopy and immunoblot analysis.

DETD Immunofluorescence microscopy of 7 .mu.m cryosections from human skeletal muscle specimens was performed as previously described for rabbit muscle. **Antibodies** used in the experiments described in this Example were from various sources. **Monoclonal antibody** (mAb) IVD31 to 50-DAG, mAb IIH6 to 156-DAG, mAbs VIA42 and XIXC2 to dystrophin were produced and characterized as described previously. The **antibodies** to dystrophin do not immunologically cross-react with spectrin, .alpha.-actin or dystrophin-related protein and furthermore stain exclusively the sarcolemma of normal human and mouse muscle cryosections, but not DMD or mdx mouse muscle **cells**, which are lacking dystrophin. Therefore the **antibodies** used in this investigation are specific probes for human dystrophin which is an important prerequisite for the diagnosis of Duchenne muscular dystrophy and related neuromuscular disorders. Highly specific **antibodies** against the dystrophin-glycoprotein complex were raised in sheep using the purified dystrophin-glycoprotein complex. **Antibodies** to the individual components of the dystrophin-glycoprotein complex were affinity-purified from individual Immobilon-P transfer membrane strips as described. While satisfactory immunoblot and immunofluorescence staining of muscle membranes and cryosections from normal and mdx mice was obtained with the **serum** taken after the first booster injection (see Example 5), human muscle membranes and cryosections were labeled much more strongly by sheep **serum** taken after a further booster injection with purified dystrophin-glycoprotein complex. Rabbit **antibodies** to the last 12 amino acids of the C-terminus of dystrophin-related protein (DRP) were previously characterized and do not immunologically cross-react with dystrophin. **Monoclonal antibody** SB-SP-1 to spectrin was purchased from Sigma Chemical Company.

DETD Depending on the secondary **antibodies** used, cryosections of skeletal muscle were pre-incubated for 20 min with 5% normal goat **serum** in PBS (50 mM sodium phosphate, pH 7.4, 0.9% NaCl) or 5% normal rabbit **serum** in PBS supplemented with 5% bovine **serum** albumin. Subsequently cryosections were treated in a 1-h incubation at 37.degree. C. with different dilutions of primary **antibody**. After washing in PBS sections were labeled at 37.degree. C. with 1:200 diluted affinity-purified fluorescein-labeled goat anti-mouse IgG (Boehringer-Mannheim) and subsequently examined in a Zeiss Axioplan fluorescence microscope. In the case of primary sheep **antibodies**, cryosections were washed in PBS and then incubated for 30 min at 37.degree. C. with 1:500 diluted biotinylated rabbit anti-sheep. . .

DETD . . . of dystrophin-associated proteins in DMD patients, all human skeletal muscle cryosections used in this investigation were characterized by labeling with **antibodies** to dystrophin and spectrin, as well as stained with wheat germ agglutinin. In contrast to dystrophin, which is completely missing in DMD skeletal muscle, it was found that the membrane cytoskeletal protein spectrin labels evenly the **cell** periphery of skeletal muscle fibers from DMD patients. Because this investigation evaluates the status of sarcolemmal glycoproteins the overall wheat. . . staining of different muscle specimens was also examined. Both normal human and DMD skeletal muscle exhibited strong WGA-labeling of the **cell** periphery, which could be specifically eliminated by pre-incubation with the competitive sugar N-acetyl-glucosamine. In addition to muscle **cell** surface staining, normal and especially DMD skeletal muscle showed strong lectin binding to the endomysial and perimysial connective **tissue**. These findings indicate that the majority of WGA-binding components of the skeletal muscle **cell** periphery are not affected in DMD. This is important in analyzing of the status of dystrophin-associated glycoproteins in DMD muscle.. . .

DETD Sheep **antibodies** to the individual components of the dystrophin-glycoprotein complex, the specificity of which was previously characterized in normal and mdx mouse. . . of dystrophin-associated proteins in muscle biopsy specimens from DMD patients. Immunofluorescence staining revealed restricted labeling of dystrophin-associated proteins to the **cell** periphery of normal

human muscle fibers. Skeletal muscle cryosections exhibited no staining of the interior of myofibers suggesting a specific. . . biopsy specimens, cryosections were all placed on the same microscopy slide, labeled with the same concentration of primary and secondary **antibodies** and were treated in an identical way during all incubation and washing steps. Undiluted affinity-purified sheep **antibodies** in combination with a biotin-streptavidin system were used for immunodetection. Photographs were taken under identical conditions with the same exposure. . . examination of DMD skeletal muscle, stained with hematoxylin and eosin, showed severe dystrophic degeneration with a rounded contour of muscle **cells**, central nucleation, a marked variability of fiber size diameter, scattered necrotic muscle fibers and increased interstitial fibrosis typical for DMD muscle. While the **cell** periphery of DMD skeletal muscle specimens exhibits normal amounts of spectrin, strong staining for WGA in interstitial connective **tissue** and a complete lack of dystrophin, it exhibits a drastic loss of 156-DAG, 59-DAP, 50-DAG, 43-DAG and 35-DAG. This was. . . of individual skeletal muscle fibers. However it should be noted that immunofluorescence staining is not only reduced, but the muscle **cell** periphery is discontinuously labeled in a patchy fashion. In stark contrast to DMD, dystrophin-associated proteins exhibited normal immunofluorescence labeling of the skeletal muscle **cell** periphery from patients suffering from limb girdle dystrophy, congenital muscular dystrophy and spinal muscular atrophy. These results demonstrate that a. . .

DETD The results obtained with affinity-purified sheep **antibodies** to 50-DAG were confirmed by immunofluorescence microscopy with **monoclonal antibody** IVD3.sub.1 against 50-DAG. A 1:100 dilution of IVD3.sub.1 hybridoma supernatant produced satisfactory results. Biopsy specimens from DMD patients of varying. . . staining intensity for 50-DAG when directly compared to normal age-matched human muscle. Similar to the results obtained with affinity-purified sheep **antibodies**, immunofluorescence staining with mAb IVD31 varied in the degree of reduction between individual DMD cases. Besides drastic reduction of immunofluorescence staining intensity, labeling of 50-DAG was observed to be discontinuous in the skeletal muscle **cell** periphery of DMD patients.

DETD . . . from a variety of other neuromuscular disorders were labeled with mAb IVD3.sub.1. 50-DAG was found in normal amounts in the **cell** periphery of muscles from patients afflicted with limb girdle dystrophy, congenital muscular dystrophy and spinal muscular atrophy. Biopsy specimens from. . . suffering from facioscapulohumeral muscular dystrophy and a patient afflicted with Friedreich's ataxia also exhibited normal immunofluorescence labeling of the muscle **cell** periphery for 50-DAG. These are important findings because they suggest that dystrophin-associated glycoproteins are not severely affected by secondary effects. . .

DETD . . . In addition, an obvious difficulty in studying dystrophin-associated glycoproteins in DMD muscle membranes was the very restricted amount of muscle **tissue** obtainable from diagnostic biopsies. This problem was overcome by acquiring 2-5 grams of DMD skeletal muscle during spinal fusion surgery. After arrival in the laboratory the **tissue** was washed in ice-cold phosphate-buffered saline and then immediately processed for centrifugation. The starting material for preparations of cardiac membranes was approximately 1 g of human heart samples. Control cardiac muscle included explanted heart **tissue** from a transplant patient and a cardiac autopsy specimen from another individual obtained shortly after death. Muscle samples were homogenized. . . for 37 min. Samples were stored in small aliquots at -135.degree. C. until use. Protein concentration was determined using bovine **serum** albumin as standard. Membrane proteins were fractionated on 3-12% gradient SDS polyacrylamide gels and transferred to nitrocellulose membranes. Immunoblot staining with **antibodies** and densitometric scanning of radioactively labeled immunoblots was performed as described previously.

DETD . . . of the dystrophin-associated proteins in DMD skeletal muscle cryosections. The fact that dystrophin-associated proteins are detectable in the DMD muscle **cell** periphery by immunofluorescence microscopy and in DMD muscle membranes by

immunoblotting suggests that remaining dystrophin-associated proteins are not present in. . .

DETD . . . DMD skeletal muscle, the status of the individual components of the dystrophin-glycoprotein complex in cardiac DMD muscle was also investigated. **Antibodies** to dystrophin-associated proteins did not exhibit satisfactory immunofluorescence labeling of human cardiac muscle thus we were not able to investigate. . .

DETD Affinity purified guinea pig polyclonal **antibodies** to the 43 kDa DAG were prepared as described by Ervasti and Campbell, **Cell** 66:1121 1131 (1991)) and used to screen 2.times.10.sup.6 clones of .lambda.gt11 expression library. Clone R43-A with a length of 600. . .

DETD . . . and three out of four potential sites for N-glycosylation. The C-terminal origin of 43 kDa DAG was confirmed using an **antibody** raised in a rabbit against a synthetic peptide corresponding to the 15 C-terminal amino acid residues of the deduced sequence. This anti-peptide **antibody** specifically recognized the 43 kDa DAG. In addition, peptide sequence determined directly from the 43 kDa DAG matched 783-793 residues. . .

DETD In order to identify the N-terminal domain of the 97 kDa precursor polypeptide, **antibodies** to different regions of the 97 kDa precursor polypeptide were produced by expressing several overlapping cDNAs encoding different regions in. . . 97 kDa precursor polypeptide. A set of pGEX vectors (Smith and Johnson, Gene 67:31-40, (1988)) were used to express various **fragments** of DNA for 97 kDa precursor protein as E. coli fusion proteins. Fusion protein-A (FP-A) contains residues 665-856 corresponding to. . . kb) into the EcoRI site of pGEX-2T. FP-C was made by ligation into the BamHI site of pGEX-1 the BamHI **fragment** of cDNA R43-C, containing C-terminal sequence with stop codon, representing last 38 amino acids. For the FP-D construct, EcoRI insert. . .

DETD Each recombinant molecule was introduced in E. coli DH5a **cells** . Overnight cultures were diluted 1:10, incubated for one hour and induced for 2 hours with 1 mM IPTG. **Cells** were resuspended in PBS and sonicated. Fusion proteins were purified from supernatant by affinity chromatography on glutathione-Sepharose (Pharmacia) and eluted with 5 mM glutathione. Dystrophin-glycoprotein complex was isolated as described Lesot et al. (**Cell** 66:1121-1131 (1991)). Sheep polyclonal **antibodies** to the purified DGC were produced as described Ohlendieck and Campbell (J. **Cell** Biol. 115:1685-1694 (1991)) and anti-fusion protein anti-bodies were affinity purified from polyclonal antiserum. A peptide representing the 15 carboxyl-terminus amino. . .

DETD Affinity-purified **antibodies** were then tested using each fusion protein and purified DGC. Consistent with the C-terminal domain encoding the 43 kDa DAG, **antibodies** to FP-A and FP-C specifically stained both bands of 43 kDa DAG doublet. However, **antibodies** to FP-B stained the 43 kDa DAG and the 156 kDa DAG components of DGC. Thus, a second product of 97 kDa precursor polypeptide appears to be the 156 kDa DAG. In accordance with this supposition, **antibodies** to FP-D stain only 156 kDa DAG. Therefore, posttranslational processing of 97 kDa precursor polypeptide gives rise to two components. . .

DETD Expression of 43/156 kDa DAG in muscle and non-muscle **tissues**

DETD **Tissue** distribution of 43/156 kDa DAG was examined by Northeastern blot analysis. Total RNA was isolated by homogenization in RNAzol (Cinna/Biotecx,. . .

DETD . . . weaker hybridizing transcript of the same size was found in brain. Northern blot analysis with total RNA from variety of **tissues**: liver, kidney, diaphragm and stomach also detected a 5.8 kb mRNA in all these **tissues**. Thus, the 5.8 kb transcript for the 43/156 kDa DAG is present in various muscle and non-muscle **tissues**, most likely originating from the same gene.

DETD Identification of the 43/156 kDa DAG in muscle and non-muscle **tissues** was performed using immunoblots of membranes from different **tissues** and affinity-purified **antibodies** to FP-B (43/156 kDa specific). Total membranes were prepared from **tissues** homogenized in 7.5 volumes of homogenization buffer (20 mM sodium pyrophosphate, 20 mM sodium phosphate monohydrate, 1 mM MgCl.sub.2, 0.3M. . .

DETD . . . for the "156 kDa" reactive protein is maybe due to differential

glycosylation of the core protein in muscle versus non-muscle **tissues**. Since the extracellular 156 kDa dystroglycan component differs in molecular weight among various **tissues**, the extracellular component has been named ".alpha.-dystroglycan" and the transmembrane component has been named ".beta.-dystroglycan" to avoid confusion.

DETD . . . of cryosections from normal and DMD skeletal muscle with 156 kDa specific (anti FP-D) and 43 kDa specific (anti FP-A) **antibodies** demonstrated a drastically reduced density of 43 kDa DAG and 156 kDa DAG in skeletal muscle of a DMD patient.. . .

DETD Since the 43/156 kDa DAG is expressed in non-muscle **tissues** we also examined expression of 43 kDa DAG in non-muscle **tissues** of control and mdx mice. The 156 kDa DAG could not be tested because polyclonal **antibodies** to the protein core of rabbit 156 kDa DAG described above do not cross react with the 156 DAG in mouse muscle. Immunoblot analysis of brain and kidney membranes from control and mdx mice, stained with polyclonal anti FP-A **antibodies** (43 kDa specific), revealed no reduction in the amount of 43 kDa DAG in these mdx **tissues**. Thus, the dramatic reduction of the 43 kDa DAG that is found in mdx mice appears to be restricted to skeletal muscle and is not found in non-muscle **tissues**.

DETD . . . A. Equivalent volumes of the resulting voids and washed Sepharose pellets were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting using **monoclonal antibody** IIH6 which is specific for the 156 kDa DAG.

DETD . . . The interaction of 156 kDa DAG with laminin was also shown by co-immunoprecipitation of laminin and 156 kDa DAG. Anti-laminin **antibodies** did not precipitate the 156 kDa DAG from alkaline extracts of rabbit skeletal muscle surface membranes. This result was consistent. . . the observation that the surface membranes used were devoid of laminin, merosin, or S-laminin as detected on immunoblots using specific **antibodies**. However, anti-laminin **antibodies** effectively precipitated the 156 kDa DAG from alkaline extracts which had been preincubated with exogenously added laminin. These results suggest. . .

DETD Cloning and **Analysis** of Human **Dystroglycan** cDNA

DETD The human gene for **dystroglycan** was characterized by genomic Southern **analysis**. DNA isolated from lymphocytes of peripheral **blood** from two individuals (DNA1 and DNA2) was digested with EcoRI, HindIII, KpnI and PstI and transferred to nitrocellulose. The blot. . .

DETD . . . KpnI, PstI were identical. Hybridization with EcoRI digested samples showed one 12 kb EcoRI band in DNA1, but two EcoRI **fragments** (9 kb and 3 kb) in DNA2, suggesting EcoRI polymorphism. The simple predictable hybridization pattern suggests the existence of a. . .

DETD . . . clones, G1 and G2 originating from DNA1 and DNA2 respectively, were chosen for further analysis. Restriction analysis demonstrated several EcoRI **fragments** within each recombinant phage, including 12 kb in G1 and 9 kb in G2 which were detected previously by Southern blot hybridization. Southern blot analysis using HD-2 cDNA as a probe identified these **fragments** specifically. The 12 kb and 9 kb EcoRI inserts were subcloned into pUC19 for further analysis and a physical map of these **fragments** was generated. Based on this map, the 12 kb EcoRI **fragment** was digested into small subclones and partially sequenced. The combination of sequencing, hybridization and PCR analysis of cloned genomic **fragments** and comparison with cDNA sequence resulted in the proposed genomic structure of the 12 kb EcoRI-**fragment** shown in FIG. 1. Interestingly, the majority of coding sequence of human dystroglycan (2.4 kb out of 2.7 kb) together. . . kb of genomic sequence. The polymorphic EcoRI site was mapped downstream of the coding region. To isolate a genomic DNA **fragment** containing exon sequence encoding the initiator methionine, the genomic library constructed from DNA1 was screened with .sup.32 P-labeled cDNA representing. . . region. Southern blot analysis of EcoRI-digested DNA1 and DNA2 with the above probe showed specific hybridization to a 6.5 kb EcoRI-**fragment**. This 6.5 kb genomic EcoRI-**fragment** (clone G3) was isolated, mapped and partially sequenced. The exon, encoding part of the 5'-untranslated region and 285 nucleotides of. . .

DETD **Tissue**-specific expression of human dystroglycan

DETD The **tissue**-specific expression of **dystroglycan** was examined by northern blot **analysis**. Radioactively labeled cDNA HD-2, which is specific to exon 2, was hybridized to 2 .mu.g mRNA from human adult muscle (skeletal and cardiac) and non-muscle **tissues** (brain, lung, liver, kidney and pancreas). The mRNA blot was prehybridized at 42.degree. C. in 5.times.SSC, 5.times.Denhardt's solution, 50% formamide,. . . and were exposed to film (X-OMAT AR, Kodak) at -80.degree. C. A band of 5.8 kb was detected in all **tissues** examined. The mRNA is most abundant in skeletal muscle and heart and less abundant in non-muscle **tissues**. mRNA from several fetal **tissues** was probed with exon 1 sequence. Interestingly, the same size **dystroglycan** specific transcript was **detected** in all **tissues** examined, which demonstrates that dystroglycan is expressed in fetal and adult muscle and non-muscle **tissues**. Since the 5.8 kb band is detected in all **tissues** examined by using probes specific for exon 1 or exon 2, it can be concluded that dystroglycan transcripts are identical in these **tissues**. In addition, RT-PCR was used to amplify skeletal-muscle specific exon 1 from adult brain and cardiac RNA using two sets. . . on the skeletal muscle cDNA were detected in brain and heart for each set of primers. This further demonstrates that **tissue** specific isoforms do not differ by the primary structure.

DETD The chromosomal localization of the **dystroglycan** gene was first **determined** by Southern blot analysis of a panel of human/hamster **cell** hybrids using a radiolabeled dystroglycan cDNA probe. Radioactively labeled dystroglycan cDNA was hybridized to a Southern blot of Bgl II-digested genomic DNA from normal human and Chinese hamster controls and 11 human x Chinese hamster somatic **cell** hybrid lines derived from six independent fusion experiments (see Franke et al., Cold Spring Harbor Symp. Quant. Biol. 51: 855-866. . .

DETD A 10 kb Bgl II human specific **fragment** was detected. This **fragment** was seen in the normal human control and in all hybrids which retained an intact copy of human chromosome 3.. . . of human chromosome 3 [i(3q)] which spontaneously arose in the subcloning of this hybrid. The 10 kb human-specific Bgl II **fragment** was not **detected** in this hybrid. Therefore, the **dystroglycan** gene locus was localized to the short arm of chromosome 3.

DETD . . . 11 metaphases with specific signals had both chromosome 3 homologs labeled, and no other chromosomes had specific signals. The somatic **cell** hybrid analysis and in situ hybridization discovered only one site of specific hybridization, which further supports the conclusion that DAG1. . .

DETD Dystrophin-Associated Protein in Autosomal Muscular Dystrophy Affected **Tissue**

DETD . . . to an autosome. It has been determined that in most cases of autosomal muscular dystrophy, dystrophin is present in affected **tissue** at near normal levels. This example discloses, however, that in muscle **tissue** from an individual afflicted with autosomal muscular dystrophy, levels of all of the dystrophin-associated proteins are substantially reduced.

DETD Muscle biopsy **tissue** from an individual afflicted with autosomal Fukuyama congenital muscular dystrophy (FCMD) was prepared in sections as described, for instance, in Examples 4 and 5. The **tissue** samples were contacted with affinity purified sheep primary **antibodies** followed by fluorescein labeled secondary **antibodies** as described previously. **Tissue** samples from normal human muscle and Duchenne muscle were similarly treated as control samples.

DETD Dystrophin and all of the dystrophin-associated glycoproteins were found to be present in normal **tissue**, but absent or substantially reduced in DMD muscle. However, in FCMD **tissue** immunostaining for all of the dystrophin-associated glycoproteins was diminished while dystrophin was not substantially reduced.

DETD . . . both males and females, 2) mode of inheritance compatible with an autosomal recessive disease, 3) North African patients, 4) elevated **serum** creatine kinase level and 5) normal expression of dystrophin in biopsied skeletal muscle analyzed by both immunohistochemistry and immunoblotting (Khurana. . .

DETD Serial transverse cryosections were immunostained with VIA4.sub.2, a **monoclonal antibody** against dystrophin, and affinity-purified sheep polyclonal **antibodies** against 156 kDa, 59 kDa, 50 kDa, 43 kDa and 35 kDa dystrophin-associated proteins as described above.

DETD In normal skeletal muscle, **antibodies** against dystrophin and dystrophin-associated proteins stained the sarcolemma. In related experiments, no abnormality of these proteins was observed in biopsy. . .

DETD Loss of the 50 kDa dystrophin-associated protein in the sarcolemma of SCARMD patients was confirmed using three other specific **antibodies** against the 50 kDa dystrophin-associated protein. These **antibodies** included a IVD3.sub.1 (a **monoclonal antibody** against the 50 kDa dystrophin-associated protein), a sheep polyclonal **antibody** affinity-purified against the 50 kDa dystrophin-associated protein peptide disclosed in SEQ ID NO:2 and an affinity-purified guinea pig polyclonal **antibody**.

DETD . . . gel so that the amount of MHC was equal for all specimens. Transfer to the nitrocellulose membrane and immunostaining with **antibodies** were performed as described above.

DETD The **antibodies** used in the staining procedure included polyclonal **antibodies** against the last 10 amino acids of dystrophin (ANTI-DYS), a **monoclonal antibody** against the 156 kDa dystrophin-associated protein (IIH6) and a mixture of affinity-purified sheep polyclonal **antibodies** against the 59 kDa, 50 kDa and 43 kDa dystrophin-associated proteins. The affinity purified sheep polyclonal **antibody** against the 35 kDa dystrophin-associated protein was not strong enough to stain the 35 kDa dystrophin-associated protein in crude muscle. . .

DETD . . . to a solid support to generate an affinity matrix. An affinity column was prepared using this matrix and a polyclonal **antibody** preparation, prepared by immunizing animals with purified dystrophin-glycoprotein complex, was passed over the column. Polyclonal **antibodies** specifically reactive with this peptide were isolated using this affinity purification method. Thus, the peptide sequence identified represents an immunogenic. . .

DETD Peptides identified in this manner can be used to immunize animals to generate **antibodies** specifically reactive with a single epitope of the 50 kDa protein. In addition, degenerate probes can be designed which can. . .

DETD Affinity-purified **antibodies** against 50 kDa dystrophin-associated glycoprotein were used to screen a rabbit skeletal muscle cDNA expression library in .lambda.gt11. An initial clone was found to contain regions of identity with sequences obtained from two proteolytic **fragments** of 50 kDa dystrophin-associated glycoprotein. This cDNA molecule was used as a probe for homology screening of rabbit and human. . .

DETD . . . been confirmed to encode 50 kDa dystrophin-associated glycoprotein by several methods. First, protein sequences were obtained from five distinct proteolytic **fragments** of 50 kDa dystrophin-associated glycoprotein, and all five are present in the deduced amino acid sequence of this clone. Second, **antibodies** affinity purified against a synthetic peptide corresponding to amino acids 354 to 363 of rabbit 50 kDa dystrophin-associated glycoprotein from sheep anti-DGC **serum** recognized the 50-kDa component of purified DGC. Third, polyclonal antiserum generated against a synthetic peptide comprised of the 15 C-terminal. . . of the deduced rabbit 50 kDa dystrophin-associated glycoprotein amino acid sequence recognizes the 50-kDa component of the purified DGC. Fourth, **antibodies** which were affinity purified against fusion protein H (FP-H) from anti-DGC guinea pig **serum** or FP-G from anti-50 kDa dystrophin-associated protein sheep **serum** recognized the 50-kDa component of purified DGC.

DETD . . . extracellular cysteines are conserved between rabbit and human 50 kDa dystrophin-associated glycoprotein. The binding of the anti-50 kDa dystrophin-associated glycoprotein **monoclonal antibody** IVD3.sub.1 on immunoblots requires nonreducing conditions, suggesting that at least one intramolecular disulfide bond is present in vivo. Based on. . .

DETD . . . transcripts were detected in bladder and small intestine,

indicating that 50 kDa dystrophin-associated glycoprotein may be expressed in smooth muscle **cells** in these **tissues**. However, immunohistochemistry or in situ hybridization will be required to precisely identify the **cell** type of origin. 50 kDa dystrophin-associated glycoprotein cDNA hybridized primarily to a band of approximately 1.5 kb, suggesting that use. . .

DETD Affinity-purified **antibodies** against 59 kDa dystrophin-associated glycoprotein were used to screen a rabbit skeletal muscle cDNA expression library in .lambda.gt11. An initial clone was found to contain regions of identity with sequences obtained from a proteolytic **fragment** of 59 kDa dystrophin-associated glycoprotein. This cDNA molecule was used as a probe for homology screening of rabbit skeletal muscle. . .

DETD . . . DNA probe corresponding to a portion of SEQ ID NO. 13 was used to mRNA blots from human skeletal muscle **cells** by Northern blot analysis. A single, strongly cross-hybridizing species was identified demonstrating that the human counterpart of the rabbit 59. .

CLM What is claimed is:

5. A prokaryotic **cell** transformed with a DNA expression construct comprising, in expressible form, a substantially pure deoxyribonucleic acid sequence encoding a mammalian 50. . .

6. A prokaryotic **cell** of claim 5 wherein the substantially pure deoxyribonucleic acid sequence is of human origin.

7. A eukaryotic **cell** transformed with a DNA expression construct comprising, in expressible form, a substantially pure deoxyribonucleic acid sequence encoding a mammalian 50. . .

8. A eukaryotic **cell** of claim 7 wherein the substantially pure deoxyribonucleic acid sequence is of human origin.

13. A prokaryotic **cell** transformed with a DNA expression construct comprising, in expressible form, a substantially pure nucleic acid molecule encoding the amino acid. . .

14. A eukaryotic **cell** transformed with a DNA expression construct comprising, in expressible form, a substantially pure nucleic acid molecule encoding the amino acid. . .

=> d bib abs 1

L58 ANSWER 1 OF 9 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 2000:933876 SCISEARCH
GA The Genuine Article (R) Number: 380KW
TI Isolation and activity of proteolytic fragment of laminin-5 alpha 3 chain
AU Tsubota Y; Mizushima H; Hirosaki T; Higashi S; Yasumitsu H; Miyazaki K (Reprint)
CS YOKOHAMA CITY UNIV, DIV CELL BIOL, KIHARA INST BIOL RES, TOTSUKA KU, 641-12 MAIOKA CHO, YOKOHAMA, KANAGAWA 244081, JAPAN (Reprint); YOKOHAMA CITY UNIV, DIV CELL BIOL, KIHARA INST BIOL RES, TOTSUKA KU, YOKOHAMA, KANAGAWA 244081, JAPAN; YOKOHAMA CITY UNIV, GRAD SCH INTEGRATED SCI, TOTSUKA KU, YOKOHAMA, KANAGAWA 244081, JAPAN
CYA JAPAN
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (30 NOV 2000) Vol. 278, No. 3, pp. 614-620.
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0006-291X.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Laminin-5 (alpha3 beta3 gamma2) is an important component of epithelial basement membranes. The 190-kDa alpha3 chain undergoes extracellular cleavage within the carboxyl (C) terminus consisting of five globular domains (G1 to G5), producing the mature laminin-5 with the 160-kDa alpha3 chain. To understand the physiological meaning of this processing, we isolated the C-terminal fragments of the alpha3 chain from the conditioned media of two kinds of human cell lines. The amino-terminal sequence of the fragments suggested that the cleavage occurs at Gln(1337)-Asp(1338) in the spacer region between the G3 and G4 domains. The G4-G5 fragment itself did not show significant activity, but it stimulated cell migration in the presence of a low concentration of the mature laminin-5, suggesting its regulatory role in cell migration. (C) 2000 Academic Press.

=> d bib abs 2

L58 ANSWER 2 OF 9 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 2001:126213 SCISEARCH
GA The Genuine Article (R) Number: 377QY
TI **Dystroglycan** facilitates correct morphogenesis in mammary epithelial cells and is highly variable among **tumor** cell lines.
AU Muschler J L (Reprint); Levy D; Campbell K; Bissell M J
CS Univ Calif Berkeley, Lawrence Berkeley Natl Lab, Berkeley, CA 94720 USA; Univ Iowa, Iowa City, IA 52242 USA
CYA USA
SO MOLECULAR BIOLOGY OF THE CELL, (DEC 2000) Vol. 11, Supp. [S], pp. 476A-476A. MA 2468.
Publisher: AMER SOC CELL BIOLOGY, 8120 WOODMONT AVE, STE 750, BETHESDA, MD 20814-2755 USA.
ISSN: 1059-1524.
DT Conference; Journal
LA English
REC Reference Count: 0

=> d bib abs 3

L58 ANSWER 3 OF 9 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
AN 2001:11324 BIOSIS
DN PREV200100011324
TI Anomalous **dystroglycan** in **carcinoma** cell lines.
AU Losasso, Carmen; Di Tommaso, Francesca; Sgambato, Alessandro; Ardito, Raffaele; Cittadini, Achille; Giardina, Bruno; Petrucci, Tamara C.; Brancaccio, Andrea (1)
CS (1) Centro Chimica dei Recettori (CNR), Istituto di Chimica e Chimica Clinica, Universita Cattolica del Sacro Cuore, Rome, 00168: a.brancaccio@uniserv.ccr.rm.cnr.it Italy
SO FEBS Letters, (10 November, 2000) Vol. 484, No. 3, pp. 194-198. print. ISSN: 0014-5793.
DT Article
LA English
SL English
AB **Dystroglycan** is a receptor responsible for crucial interactions between extracellular matrix and cytoplasmic space. We provide the first evidence that **dystroglycan** is truncated. In HC11 normal murine and the 184B5 non-**tumorigenic** mammary human cell lines, the expected beta-**dystroglycan** 43 kDa band was found but human breast T47D, BT549, MCF7, colon HT29, HCT116, SW620, prostate DU145 and cervical HeLa **cancer** cells expressed an anomalous apprxeq31 kDa beta-**dystroglycan** band. alpha-**dystroglycan** was udetectable in most of the cell lines in which beta-**dystroglycan** was found as a apprxeq31 kDa species. An anomalous apprxeq31 kDa beta-**dystroglycan** band was also observed in N-methyl-N-nitrosurea-induced primary rat mammary tumours. Reverse transcriptase polymerase chain reaction experiments confirmed the absence of alternative splicing events and/or expression of eventual **dystroglycan** isoforms. Using protein extraction procedures at low- and high-ionic strength, we demonstrated that both the 43 kDa and apprxeq31 kDa beta-**dystroglycan** bands harbour their transmembrane segment.

=> d bib abs 4

L58 ANSWER 4 OF 9 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1999-395091 [33] WPIDS
DNC C1999-116130
TI New compositions for increasing survival of transplanted cells.
DC B04 D16
IN TREMBLAY, J P
PA (UYLA-N) UNIV LAVAL
CYC 84
PI WO 9930730 A1 19990624 (199933)* EN 89p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD
GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV
MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT
UA UG US UZ VN YU ZW
AU 9918649 A 19990705 (199948)
ADT WO 9930730 A1 WO 1998-CA1176 19981215; AU 9918649 A AU 1999-18649 19981215
FDT AU 9918649 A Based on WO 9930730
PRAI CA 1997-2225837 19971224; CA 1997-2224768 19971215
AN 1999-395091 [33] WPIDS
AB WO 9930730 A UPAB: 19990819
NOVELTY - A novel composition for increasing the survival of transplanted cells upon their transplantation or injection into a host is new.

DETAILED DESCRIPTION - A novel composition for increasing the survival of transplanted cells upon their transplantation or injection comprises an anti-inflammatory agent which interferes with the recruitment, the binding or the activation of pro-inflammatory cells of the host toward the cells, so as to prevent the destruction of the transplanted cells by the host, with the proviso that the composition does not consist of an anti-LFA-1 antibody or anti-ICAM-1 antibody fragment, and a carrier.

USE - The anti-inflammatory agents hinder the binding of pro-inflammatory cells to transplanted cells or inhibit the recruitment of pro-inflammatory cells on the transplanted cells. The compositions can be used to treat e.g. Duchenne or Becker muscular dystrophy, inflammatory disease such as arthritis or sporiasis, heart insufficiency, nanism, hemophilia or Parkinson's disease.

Dwg.0/8

=> d kwic 4

L58 ANSWER 4 OF 9 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
TECH. . .
platelet derived growth factor (PDGF), hepatocyte growth factor 1 scatter factor (HGF/SF), heparin binding epidermal growth factor-like growth factor (HB-EGF), **tumor** necrosis factor (TNF)-alpha or transferin. The transplanted cells may be genetically engineered to express a gene which is capable of. . . or RU-486. The transplanted cells may be genetically engineered to express a functional protein, e.g. dystrophin, utrophin, a sarcoglycan, a **dystroglycan**, a syntrophin, a sarcospan, merosine or a metabolic enzyme, a coagulation factor, a hormone or growth factor, myotonine kinase, tyrosine. . .

=> d bib abs 5

L58 ANSWER 5 OF 9 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 1999:902032 SCISEARCH
GA The Genuine Article (R) Number: 241JQ
TI Reduced expression of **dystroglycan** in prostate and breast **cancer**.
AU Henry M D (Reprint); Cohen M B; Durbeej M; Campbell K P
CS UNIV IOWA, DEPT PHYSIOL & BIOPHYS, IOWA CITY, IA; UNIV IOWA, DEPT PATHOL, IOWA CITY, IA
CYA USA
SO AMERICAN JOURNAL OF HUMAN GENETICS, (OCT 1999) Vol. 65, No. 4, Supp. [S], pp. 696-696.
Publisher: UNIV CHICAGO PRESS, 5720 SOUTH WOODLAWN AVE, CHICAGO, IL 60637-1603.
ISSN: 0002-9297.
DT Conference; Journal
FS LIFE; CLIN
LA English
REC Reference Count: 0.

=> d bib abs 6

L58 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1999:497447 BIOSIS
DN PREV199900497447
TI Reduced expression of **dystroglycan** in prostate and breast **cancer.**
AU Henry, M. D. (1); Cohen, M. B.; Durbeej, M. (1); Campbell, K. P. (1)
CS (1) HHMI, Department of Physiology and Biophysics, Neurology, University of Iowa, Iowa City, IA USA
SO American Journal of Human Genetics, (Oct., 1999) Vol. 65, No. 4, pp. A130. Meeting Info.: 49th Annual Meeting of the American Society of Human Genetics San Francisco, California, USA October 19-23, 1999 The American Society of Human Genetics
. ISSN: 0002-9297.
DT Conference
LA English

=> d bib abs 7

L58 ANSWER 7 OF 9 MEDLINE
AN 1999059799 MEDLINE
DN 99059799 PubMed ID: 9841899
TI Identification of laminin-10/11 as a strong cell adhesive complex for a normal and a malignant human epithelial cell line.
AU Ferletta M; Ekblom P
CS Department of Animal Physiology, Uppsala University Biomedical Center, BOX 596, SE-75124 Uppsala, Sweden.
SO JOURNAL OF CELL SCIENCE, (1999 Jan) 112 (Pt 1) 1-10.
Journal code: HNK; 0052457. ISSN: 0021-9533.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199905
ED Entered STN: 19990607
Last Updated on STN: 19990607
Entered Medline: 19990526
AB Laminins are heterotrimeric proteins of basement membranes. More than 50 different trimers may exist. Laminin-10 (alpha5beta1gamma1 rather than laminin-1 (alpha1beta1gamma1) could be the most abundant isoform in the adult stage, and laminin-10 is made by several developing epithelial sheets. We show here that a much used commercial human preparation contains laminin-10 (alpha5beta1gamma1), some laminin-11 (alpha5beta2gamma1), but no laminin-1. Moreover, the laminin-10/11 mixture was found to be a strong adhesive for two human cell lines derived from epithelia. Antibodies against integrin beta1, alpha6 or alpha3 (at 50 microgram/ml) or **dystroglycan** did not inhibit cell attachment to laminin-10/11, although lower concentrations of anti-**dystroglycan** and integrin alpha6 antibodies inhibited cell binding to laminin-1.

=> d bib abs 8

L58 ANSWER 8 OF 9 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 1998:831683 SCISEARCH
GA The Genuine Article (R) Number: 132RN
TI Structural organization and chromosomal localization of Hyal2, a gene encoding a lysosomal hyaluronidase
AU Strobl B; Wechselberger C; Beier D R; Lepperdinger G (Reprint)
CS AUSTRIAN ACAD SCI, INST MOL BIOL, DEPT BIOCHEM, BILLROTHSTR 11, A-5020 SALZBURG, AUSTRIA (Reprint); AUSTRIAN ACAD SCI, INST MOL BIOL, DEPT BIOCHEM, A-5020 SALZBURG, AUSTRIA; BRIGHAM & WOMENS HOSP, DIV GENET, BOSTON, MA 02115
CYA AUSTRIA; USA
SO GENOMICS, (15 OCT 1998) Vol. 53, No. 2, pp. 214-219.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0888-7543.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 35
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB The human HYAL2 gene encodes a lysosomal hyaluronidase that is related to the testicular PH-20 hyaluronidase, Regions conserved in these proteins have been used to design PCR primers suitable for the isolation of a fragment of the murine Hyal2 gene. This fragment was used to isolate the Hyal2 cDNA from a cDNA library. The cloned cDNA has an open reading frame of 473 codons and a 3'-untranslated region of 302 bases plus a poly(A) tail. Using this cDNA, the corresponding genomic DNA was characterized from 129SVJ mice. The murine Hyal2 gene is approximately 3.5 kb, contains the coding sequence for the mRNA on four exons, and is localized on chromosome 9 between the microsatellite markers D9Mit183 and D9Mit17 near the genes for **dystroglycan** and transferrin. The gene is expressed ubiquitously, the sole exception being adult brain. (C) 1998 Academic Press.

=> d bib abs 9

L58 ANSWER 9 OF 9 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 93:643592 SCISEARCH
GA The Genuine Article (R) Number: MC223
TI HUMAN **DYSTROGLYCAN** - SKELETAL-MUSCLE CDNA, GENOMIC STRUCTURE, ORIGIN OF TISSUE-SPECIFIC ISOFORMS AND CHROMOSOMAL LOCALIZATION
AU IBRAGHIMOVBESKROVNAYA O; MILATOVICH A; OZCELIK T; YANG B; KOEPNICK K; FRANCKE U; CAMPBELL K P (Reprint)
CS UNIV IOWA, COLL MED, HOWARD HUGHES MED INST, 400 EMRB, IOWA CITY, IA, 52242; UNIV IOWA, COLL MED, DEPT PHYSIOL & BIOPHYS, IOWA CITY, IA, 52242; STANFORD UNIV, MED CTR, SCH MED, HOWARD HUGHES MED INST, STANFORD, CA, 94305; STANFORD UNIV, MED CTR, SCH MED, DEPT GENET & PEDIAT, STANFORD, CA, 94305
CYA USA
SO HUMAN MOLECULAR GENETICS, (OCT 1993) Vol. 2, No. 10, pp. 1651-1657. ISSN: 0964-6906.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 33
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB **Dystroglycan** is a novel laminin binding component of the dystrophin-glycoprotein complex which provides a linkage between the subsarcolemmal cytoskeleton and the extracellular matrix. Here we report the cDNA and genomic structure of human **dystroglycan**. The human **dystroglycan** is encoded by a single gene (DAG1) mapped to chromosome 3 band p21. The coding sequence is organized into two exons, separated by a large intron. The predicted amino acid sequence of human and rabbit **dystroglycan** are 93% identical with predicted glycosylation sites being conserved. Human **dystroglycan** is expressed in a variety of fetal and adult tissues. Our data suggest that muscle and non-muscle isoforms of **dystroglycan** differ by carbohydrate moieties but not protein sequence. Therefore, we hypothesize that variable glycosylation of the conserved protein core might modulate laminin binding. The relationship of **dystroglycan** to human diseases is discussed.

=> d kwic 9

L58 ANSWER 9 OF 9 SCISEARCH COPYRIGHT 2001 ISI (R)
TI HUMAN **DYSTROGLYCAN** - SKELETAL-MUSCLE CDNA, GENOMIC STRUCTURE, ORIGIN OF TISSUE-SPECIFIC ISOFORMS AND CHROMOSOMAL LOCALIZATION
AB **Dystroglycan** is a novel laminin binding component of the dystrophin-glycoprotein complex which provides a linkage between the subsarcolemmal cytoskeleton and the extracellular matrix. Here we report the cDNA and genomic structure of human **dystroglycan**. The human **dystroglycan** is encoded by a single gene (DAG1) mapped to chromosome 3 band p21. The coding sequence is organized into two exons, separated by a large intron. The predicted amino acid sequence of human and rabbit **dystroglycan** are 93% identical with predicted glycosylation sites being conserved. Human **dystroglycan** is expressed in a variety of fetal and adult tissues. Our data suggest that muscle and non-muscle isoforms of **dystroglycan** differ by carbohydrate moieties but not protein sequence. Therefore, we hypothesize that variable glycosylation of the conserved protein core might modulate laminin binding. The relationship of **dystroglycan** to human diseases is discussed.
STP KeyWords Plus (R): EPIDERMOLYSIS BULLOSA SIMPLEX; DUCHENNE MUSCULAR-DYSTROPHY; PROTEOGLYCAN CORE PROTEIN; RENAL-CELL **CARCINOMA**; GLYCOPROTEIN; MEMBRANE; RECEPTOR; HETEROZYGOSITY; ORGANIZATION; DEFICIENCY

09/652,493

Set	Items	Description
S1	0	DYSTOGLYCAN
S2	950	DYSTROGLYCAN
S3	12	S2 AND (CANCER? TUMOR? OR NEOPLASM?)
S4	6	RD (unique items) **4/9/3 (Item 3 from file: 5)**

DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

12804175 BIOSIS NO.: 200100011324
Anomalous dystroglycan **in carcinoma cell lines.**
AUTHOR: Losasso Carmen; Di Tommaso Francesca; Sgambato Alessandro; Ardito Raffaele; Cittadini Achille; Giardina Bruno; Petrucci Tamara C; Brancaccio Andrea(a)
AUTHOR ADDRESS: (a)Centro Chimica dei Recettori (CNR), Istituto di Chimica e Chimica Clinica, Universita Cattolica del Sacro Cuore, Rome, 00168: a.brancaccio@uniserv.ccr.rm.cnr.it**Italy
JOURNAL: FEBS Letters 484 (3):p194-198 10 November, 2000
MEDIUM: print
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: **Dystroglycan** is a receptor responsible for crucial interactions between extracellular matrix and cytoplasmic space. We provide the first evidence that **dystroglycan** is truncated. In HC11 normal murine and the 184B5 non-tumorigenic mammary human cell lines, the expected beta-**dystroglycan** 43 kDa band was found but human breast T47D, BT549, MCF7, colon HT29, HCT116, SW620, prostate DU145 and cervical HeLa cancer cells expressed an anomalous apprxeq31 kDa beta- **dystroglycan** band. alpha-**dystroglycan** was udetectable in most of the cell lines in which beta-**dystroglycan** was found as a apprxeq31 kDa species. An anomalous apprxeq31 kDa beta- **dystroglycan** band was also observed in N-methyl-N-nitrosurea-induced primary rat mammary tumours. Reverse transcriptase polymerase chain reaction experiments confirmed the absence of alternative splicing events and/or expression of eventual **dystroglycan** isoforms. Using protein extraction procedures at low- and high-ionic strength, we demonstrated that both the 43 kDa and apprxeq31 kDa beta- **dystroglycan** bands harbour their transmembrane segment.

4/9/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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12202598 BIOSIS NO.: 199900497447
Reduced expression of dystroglycan **in prostate and breast cancer.**
AUTHOR: Henry M D(a); Cohen M B; Durbeej M(a); Campbell K P(a)
AUTHOR ADDRESS: (a)HHMI, Department of Physiology and Biophysics, Neurology, University of Iowa, Iowa City, IA**USA
JOURNAL: American Journal of Human Genetics 65 (4):pA130 Oct., 1999
CONFERENCE/MEETING: 49th Annual Meeting of the American Society of Human Genetics San Francisco, California, USA October 19-23, 1999
SPONSOR: The American Society of Human Genetics
ISSN: 0002-9297
RECORD TYPE: Citation